

=> d his ful

FILE 'WPIX' ENTERED AT 12:32:50 ON 03 JUL 2006

L1	141	SEA ABB=ON	PLU=ON	MD2 OR MD 2
L2	3272	SEA ABB=ON	PLU=ON	ENDOTOXIN# OR ENDO (2W) TOXIN#
L3	6	SEA ABB=ON	PLU=ON	L1 AND L2
		D TI 1-6		
		D .WP 4		
L4	10586	SEA ABB=ON	PLU=ON	TOXIN#
L5	1	SEA ABB=ON	PLU=ON	L4 AND L1
L6	6	SEA ABB=ON	PLU=ON	L5 OR L3
L7	355	SEA ABB=ON	PLU=ON	WEISS J?/AU
L8	1	SEA ABB=ON	PLU=ON	GIOANNINI T?/AU
L9	1	SEA ABB=ON	PLU=ON	TEGHANEMT A?/AU
L10	316	SEA ABB=ON	PLU=ON	SUBRAMANIAN R?/AU
L11	670	SEA ABB=ON	PLU=ON	(L7 OR L8 OR L9 OR L10)
L12	1	SEA ABB=ON	PLU=ON	L11 AND L1
L13	2	SEA ABB=ON	PLU=ON	L11 AND L2
L14	2	SEA ABB=ON	PLU=ON	L12 OR L13
L15	1	SEA ABB=ON	PLU=ON	L14 NOT L3

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=> fil wpix
FILE 'WPIX' ENTERED AT 12:35:47 ON 03 JUL 2006
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FILE LAST UPDATED: 29 JUN 2006 <20060629/UP>
MOST RECENT DERWENT UPDATE: 200641 <200641/DW>
DERWENT WORLD PATENTS INDEX SUBSCRIBER FILE, COVERS 1963 TO DATE

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=> d que 16
L1      141 SEA FILE=WPIX ABB=ON PLU=ON MD2 OR MD 2
L2      3272 SEA FILE=WPIX ABB=ON PLU=ON ENDOTOXIN# OR ENDO (2W) TOXIN#
L3       6 SEA FILE=WPIX ABB=ON PLU=ON L1 AND L2
L4     10586 SEA FILE=WPIX ABB=ON PLU=ON TOXIN#
L5        1 SEA FILE=WPIX ABB=ON PLU=ON L4 AND L1
L6        6 SEA FILE=WPIX ABB=ON PLU=ON L5 OR L3

=> d que 115
L1      141 SEA FILE=WPIX ABB=ON PLU=ON MD2 OR MD 2
L2      3272 SEA FILE=WPIX ABB=ON PLU=ON ENDOTOXIN# OR ENDO (2W) TOXIN#
L3       6 SEA FILE=WPIX ABB=ON PLU=ON L1 AND L2
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L8        1 SEA FILE=WPIX ABB=ON PLU=ON GIOANNINI T?/AU
L9        1 SEA FILE=WPIX ABB=ON PLU=ON TEGHANEMT A?/AU
L10     316 SEA FILE=WPIX ABB=ON PLU=ON SUBRAMANIAN R?/AU
L11     670 SEA FILE=WPIX ABB=ON PLU=ON (L7 OR L8 OR L9 OR L10)
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L13        2 SEA FILE=WPIX ABB=ON PLU=ON L11 AND L2
L14        2 SEA FILE=WPIX ABB=ON PLU=ON L12 OR L13
L15        1 SEA FILE=WPIX ABB=ON PLU=ON L14 NOT L3

=> d .wp 16 1-6;d bib ab 115

L6 ANSWER 1 OF 6 WPIX COPYRIGHT 2006 THE THOMSON CORP on STN
AN 2006-253953 [26] WPIX
DNC C2006-082768
TI New soluble Toll-like receptor 4 protein, useful as a therapeutic agent
for treating endotoxin-induced inflammation.
DC B04 D16
IN HYAKUSHIMA, N; KUROKI, Y; MITSUZAWA, H
PA (NISC-N) JAPAN SCI & TECHNOLOGY AGENCY
CYC 112
PI WO 2006033481 A1 20060330 (200626)* JA 78
```

RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IS IT KE LS LT LU LV MC MW MZ NA NL OA PL PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KM KP KR KZ LC LK LR LS LT LU LV LY MA MD MG MK MN MW MX MZ NA NG NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SM SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW

ADT WO 2006033481 A1 WO 2005-JP18207 20050922

PRAI JP 2004-277421 20040924

AB WO2006033481 A UPAB: 20060421

NOVELTY - A soluble Toll-like receptor 4 protein (I) (TLR4), comprising an amino acid sequence having a fully defined 608 amino acid (SEQ ID No: 1) sequence, given in the specification, or an amino acid sequence that is substantially the same as SEQ ID Number 1, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

- (1) DNA (II) encoding (I);
- (2) recombinant vector (III) comprising (II);
- (3) non-human host cell (IV) transformed with (III);
- (4) preparing (I); and
- (5) therapeutic agent (A1) comprising (I).

ACTIVITY - Antiinflammatory. In vivo analysis of soluble Toll-like receptor 4 protein (sTLR4) and MD-2 in suppressing inflammation induced by endotoxin was carried out as follows. A female BALB/C mouse was anesthetized by ketamine HCl and xylazine hydrochloride. The mice injected with sTLR4 and MD-2 protein were taken as a test group, and mice injected with sTLR2 was taken as control group. Lipopolysaccharide (LPS) (1 mu g) was dripped in trachea. After 16 hours, 1 ml of Hank's solution was used to wash bronchus alveolus. The concentration of tumor necrosis factor alpha in bronchus alveolus was measured. Results showed that inflammation induced by endotoxin was significantly reduced in test group.

MECHANISM OF ACTION - None given.

USE - (I) Or (A1) is useful for treating endotoxin induced inflammation (claimed), inflammation induced by nuclear factor kappa B activation, and interleukin-8 secretion.

DESCRIPTION OF DRAWING(S) - The figure is a graph representing the inflammatory cytokine of lung decreased by administration of soluble Toll-like receptor 4 protein and MD-2.

Dwg.9/9

TECH UPTX: 20060421

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preparation: (I) Is prepared by culturing (IV), and expressing (I) (claimed).

Preferred Protein: (I) Comprises an amino acid sequence having SEQ ID No: 1, in which one or more amino acids are substituted, inserted, deleted or added. (I) Comprises SEQ ID No: 1.

Preferred Host Cell: (IV) Is of insect cell origin.

Preferred Agent: (A1) Is an antiinflammatory agent for treatment of endotoxin-induced inflammation.

L6 ANSWER 2 OF 6 WPIX COPYRIGHT 2006 THE THOMSON CORP on STN
AN 2006-212808 [22] WPIX

DNC C2006-070123

TI New mutant or truncated MD-2 polypeptide capable of specifically binding to lipopolysaccharide but having decreased binding affinity to toll-like receptor-4, for treating e.g. sepsis.

DC B04 D16

IN KIRKLAND, T N; VIRIYAKOSOL, S

PA (REGC) UNIV CALIFORNIA

CYC 111

PI WO 2006025995 A2 20060309 (200622)* EN 58

RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IS IT
 KE LS LT LU LV MC MW MZ NA NL OA PL PT RO SD SE SI SK SL SZ TR TZ
 UG ZM ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE
 DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG
 KM KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NG NI
 NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SM SY TJ TM TN TR TT
 TZ UA UG US UZ VC VN YU ZA ZM ZW

ADT WO 2006025995 A2 WO 2005-US26771 20050727

PRAI US 2005-681097P 20050513; US 2004-591805P 20040727

AB WO2006025995 A UPAB: 20060331

NOVELTY - A mutant or truncated **MD-2** polypeptide (P1), where (P1) specifically binds lipopolysaccharide (LPS) but cannot bind or has decreased binding affinity to toll-like receptor-4 (TLR4), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

- (1) a chimeric protein (P2) comprising a first domain having an **MD-2** polypeptide and at least a second domain;
- (2) a pharmaceutical composition (PC) comprising (P1) or (P2) and a excipient;
- (3) a parenteral formulation (I) comprising (P1) or (P2);
- (4) an enteral formulation (II) comprising (P1) or (P2);
- (5) decreasing (M1) the amount of **endotoxin** in a biological fluid, comprising providing a composition or formulation comprising (P1) or (P2), and administering the composition or formulation to a subject in need of it, thus decreasing the amount of **endotoxin** in the biological fluid;
- (6) an isolated or recombinant nucleic acid (N1) encoding (P1) or (P2);
- (7) a vector or expression cassette (V1) comprising (N1);
- (8) a host cell (H1) comprising (V1);
- (9) a non-human transgenic animal comprising (V1) or (N1);
- (10) a composition (C1) for transfecting nucleic acids into a cell, comprising (P1), and/or (P2), LPS and a nucleic acid; and
- (11) transfecting (M2) a cell with nucleic acid, comprising providing a nucleic acid-comprising (C1), and contacting the cell with (C1) under conditions where (C1) is internalized into the cell.

ACTIVITY - Antibacterial; Immunomodulatory; Antiinflammatory; Respiratory-Gen.; Antiasthmatic; Virucide; Antiparasitic; Fungicide; Vulnerary; Antibacterial. No supporting data is given.

MECHANISM OF ACTION - Decreases **endotoxin** amount (claimed).

USE - (P1), (P2), (I) Or (II) is useful for decreasing the amount of **endotoxin** in a biological fluid, where the biological fluid comprises blood, serum or cerebrospinal fluid (CSF). PC Is useful for treating or ameliorating sepsis, which involves providing PC, and administering PC to a subject in need of it, thus treating the sepsis. PC Is useful for treating or ameliorating a condition comprising an LPS-induced disease, infection or pathology, which involves administering PC, where the LPS-induced disease or pathology is chosen from **endotoxin**-induced septic shock, **endotoxin**-induced toxic shock, sepsis, sever sepsis, septic shock caused by Gram-negative bacteria, bacterial infections, shock inflammatory diseases, graft versus host disease, autoimmune diseases, acute respiratory distress syndrome, granulomatous diseases, chronic infections, transplant rejection, acute respiratory asthma, viral infections, parasitic infections, fungal infections and trauma (all claimed). (P1) Is useful as a bacteria-targeting agent to treat sepsis caused by infectious diseases, such as bacterial and fungal diseases. (P1) Or (P2) is useful for

increasing clearance of LPS from circulation.

DESCRIPTION OF DRAWING(S) - The figure is a graph representing the effect of cell surface level of **MD-2** polypeptide on lipopolysaccharide activation.

Dwg.1/10

TECH

UPTX: 20060331

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Polypeptide: (P1) Is a variant of the human **MD-2** having a fully defined 160 amino acid (SEQ ID No: 1) sequence, given in the specification, or a mature form of it lacking a signal sequence. (P1) Has any one of a fully defined 144 amino acid (SEQ ID No: 37, 38 or 39) sequence, given in the specification, or an amino acid sequence where one, several or all of the alanines in SEQ ID No: 1 or one, several or all of the alanines in a polyA domain of SEQ ID No: 37, 38 or 39 are replaced with a valine, leucine, an isoleucine or another aliphatic amino acid, or glycine, or equivalents, or their combination. (P1) Comprises a naturally secreted form of human **MD-2**, and is glycosylated. (P1) Comprises a dimeric **MD-2**, and an amino terminal fragment of the **MD-2** polypeptide. (P1) Comprises a peptidomimetic or synthetic protein. (P2) Comprises a recombinant fusion protein. The second domain comprises an opsinizing agent, where the opsinizing agent comprises an antibody Fc domain or an antibody that binds to an Fc receptor. (P2) Comprises two or more antibody Fc domains, or two or more **MD-2** polypeptides. The opsinizing agent comprises a human opsinizing agent. The **MD-2** polypeptide comprises a mature **MD-2** lacking a signal sequence, and a human **MD-2** polypeptide. The **MD-2** polypeptide comprises a mutant or truncated **MD-2** polypeptide, where the mutant or truncated polypeptide specifically binds LPS and binds TLR4 with an affinity less than that of wild type TLR4. The mutant or truncated polypeptide does not bind wild type TLR4. The **MD-2** variant has increased affinity for LPS, or TLR4 or CD14. The **MD-2** polypeptide comprises a mutation or truncation of a human **MD-2** polypeptide. (P2) Is soluble in aqueous media or the **MD-2** polypeptide comprises a naturally secreted form of human **MD-2**. (P2) Is glycosylated, and the **MD-2** polypeptide comprises a dimeric **MD-2**. The **MD-2** polypeptide comprises an amino terminal fragment of the **MD-2** polypeptide. The second domain comprises an Fc domain, a protein C, an antibacterial or bacteriostatic peptide or protein, antibiotic, a cytokine, an immunoregulatory agent, anti-inflammatory agent, a complement activating agent, carbohydrate-binding domain or their combination. The protein C is a human activated protein C. The complement activating agent comprises a collagen-like domain, fibrinogen-like domain or ficolin. (P2) Is a recombinant protein, and comprises a peptidomimetic or synthetic protein. The first domain is joined to the second domain by a chemical linking agent.

Preferred Host Cell: (H1) Is a bacterial cell, mammalian cell, fungal cell, an insect cell, a yeast cell or plant cell.

Preferred Composition: In (C1), the nucleic acid comprises:

- (a) naked DNA or RNA, and optionally the naked DNA is operably linked to a promoter; or
- (b) plasmid DNA, an expression cassette or expression vector.

The LPS comprises a bacterial LPS. (C1) Further comprises a lipopolysaccharide-binding protein (LBP) and/or a CD14 polypeptide.

Preferred Method: In (M2), the transfecting is an *in vivo* transfection or *in vitro* transfection. The cell is a bacterial cell or mammalian cell, where optionally the mammalian cell is a human cell.

L6 ANSWER 3 OF 6 WPIX COPYRIGHT 2006 THE THOMSON CORP on STN
 AN 2005-372350 [38] WPIX
 DNN N2005-301093 DNC C2005-115401
 TI New anti-TLR4-MD-2 monoclonal antibody not exerting effect of B-cell proliferation inhibition and TNF production inhibition in macrophages, through in vitro lipopolysaccharide stimulation, for treating **endotoxin** shock.
 DC B04 D16 S03
 IN MIYAKE, K; TAKAMURA, S
 PA (NISC-N) JAPAN SCI & TECHNOLOGY AGENCY
 CYC 108
 PI WO 2005047330 A1 20050526 (200538)* JA 30
 RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE
 LS LU MC MW MZ NA NL OA PL PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
 W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE
 DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG
 KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ
 OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG
 US UZ VC VN YU ZA ZM ZW
 ADT WO 2005047330 A1 WO 2004-JP14194 20040928
 PRAI JP 2003-387173 20031117
 AB WO2005047330 A UPAB: 20050616
 NOVELTY - A monoclonal antibody (I) capable of specifically recognizing a TLR4-MD-2 composite and not exerting effect of B-cell proliferation inhibition and TNF production inhibition in macrophages, through in vitro lipopolysaccharide (LPS) stimulation, is new.
 DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
 (1) an anti-mouse TLR4-MD-2 monoclonal antibody Sa 15-21 capable recognizing an antigenic determinant of mouse TLR4 in a mouse TLR4-MD-2 composite, in the N-terminal end;
 (2) an anti-human TLR4 monoclonal antibody TF904 capable of specifically recognizing the antigenic determinant of human TLR4, in the N-terminal end;
 (3) a hybridoma capable of producing an anti-human TLR4 monoclonal antibody TF904 which specifically recognizes an antigenic determinant of human TLR4 (FER ABP-10118), in the N-terminal end;
 (4) a therapeutic agent (A1) of **endotoxin** shock comprising (I); and
 (5) screening an agent capable of promoting **endotoxin** shock inhibitory effect or substance inhibiting **endotoxin** shock, comprising administering anti-TLR4-MD-2 mouse monoclonal antibody Sa 15-21 capable of recognizing TLR4-MD-2 composite and a test substance, and evaluating the grade of **endotoxin** shock in a mouse, before and after the **endotoxin** shock.
 ACTIVITY - Antibacterial; Immunosuppressive. No supporting data is given.
 MECHANISM OF ACTION - TRL4-MD-2 composite antagonist.
 USE - (I) Is useful for treating or preventing **endotoxin** shock (claimed).
 ADVANTAGE - (I) Effectively treats **endotoxin** shock.
 Dwg.0/8
 TECH UPTX: 20050616
 TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Antibody: (I) Enhances TNF production to **endotoxin** shock. (I) Recognizes an antigenic determinant of TLR4 in a TLR4-MD-2 composite, in the N-terminal end.

L6 ANSWER 4 OF 6 WPIX COPYRIGHT 2006 THE THOMSON CORP on STN
 AN 2005-354786 [36] WPIX
 DNC C2005-109712

TI New purified complexes comprising **endotoxin** bound to **MD-2**, useful for promoting innate immune response, as immunological adjuvants, or for treating conditions associated with **endotoxin**-mediated cell activation, e.g. sepsis.

DC B04 D16

IN GIOANNINI, T L; SUBRAMANIAN, R; TEGHANEMT, A; WEISS, J P
 PA (GIOA-I) GIOANNINI T L; (SUBR-I) SUBRAMANIAN R; (TEGH-I) TEGHANEMT A;
 (WEIS-I) WEISS J P; (IOWA) UNIV IOWA RES FOUND

CYC 108

PI US 2005106179 A1 20050519 (200536)* 34
 WO 2005049067 A1 20050602 (200536) EN

RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IS IT
 KE LS LU MC MW MZ NA NL OA PL PT RO SD SE SI SK SL SZ TR TZ UG ZM
 ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE
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 KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ
 OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG
 US UZ VC VN YU ZA ZM ZW

ADT US 2005106179 A1 US 2003-715876 20031117; WO 2005049067 A1 WO 2004-US38375
 20041117

PRAI US 2003-715876 20031117

AB US2005106179 A UPAB: 20050608
 NOVELTY - A purified complex comprising **endotoxin** bound to **MD-2**, is new.
 ACTIVITY - Antibacterial; Immunosuppressive; Hepatotropic;
 Gastrointestinal-Gen.; Antiinflammatory; CNS-Gen.; Respiratory-Gen.;
 Antiasthmatic; Cytostatic.
 No biological data given.
 MECHANISM OF ACTION - Gene therapy; Vaccine.
 USE - The complex is useful for decreasing undesirable **endotoxin**-mediated inflammation, or for promoting innate immune response and as immunological adjuvants. The complex and methods may be used for treating conditions associated with **endotoxin**-mediated cell activation, such as sepsis, liver disease, inflammatory bowel disease, cystic fibrosis, asthma, autoimmune diseases, cancer or bacterial infections.

Dwg.0/14

TECH UPTX: 20050608
 TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Complex: The **endotoxin** is a wild-type **endotoxin** or a gram-negative bacterial **endotoxin**. The gram-negative bacterium is a *Neisseria*, *Escherichia*, *Pseudomonas*, *Hemophilus*, *Salmonella* or *Francisella* bacterium. The bacterium is preferably *Neisseria meningitidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Hemophilus influenzae*, *Salmonella typhimurium* or *Francisella tularensis*. The complex has a molecular weight of about 25000. It consists essentially of one molecule of **endotoxin** bound to one molecule of **MD-2**. The complex is soluble in water. It binds to Toll-like receptor 4 (TLR4) and produces TLR4-dependent activation of cells. The complex produces a half maximal TLR4-dependent activation of cells at a concentration of less than 1 nM of the complex, particularly about 30 pM or less of the complex. The **endotoxin** is hexa-acylated. It is an under-acylated **endotoxin**, a tetra-acylated **endotoxin** or a penta-acylated **endotoxin**. The complex produces less TLR4-dependent activation of cells as compared to a complex comprising an **endotoxin** that is hexa-acylated. A composition comprises the complex and a pharmaceutical carrier.

L6 ANSWER 5 OF 6 WPIX COPYRIGHT 2006 THE THOMSON CORP on STN
 AN 2004-143114 [14] WPIX
 DNN N2004-114058 DNC C2004-057716
 TI Extracorporeal adsorption agent for removing harmful substances that induce sepsis, by treating blood obtained from mammal by passing blood through adsorption column assembly at flow rate that fluidized bed of particles is formed.
 DC B04 S03
 IN HEEGAARD, P M H; LIHME, A O F
 PA (UPFR-N) UPFRONT CHROMATOGRAPHY AS
 CYC 105
 PI WO 2004008138 A2 20040122 (200414)* EN 56
 RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS
 LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
 W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
 DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
 KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PG PH
 PL PT RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN
 YU ZA ZM ZW
 AU 2003242509 A1 20040202 (200450)
 EP 1521624 A2 20050413 (200525) EN
 R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LT LU LV
 MC MK NL PT RO SE SI SK TR
 JP 2005532130 W 20051027 (200571) 48
 US 2005249724 A1 20051110 (200574)
 AU 2003242509 A8 20051103 (200629)
 ADT WO 2004008138 A2 WO 2003-DK483 20030709; AU 2003242509 A1 AU 2003-242509
 20030709; EP 1521624 A2 EP 2003-763618 20030709, WO 2003-DK483 20030709;
 JP 2005532130 W WO 2003-DK483 20030709, JP 2004-520339 20030709; US
 2005249724 A1 WO 2003-DK483 20030709, US 2005-520685 20050527; AU
 2003242509 A8 AU 2003-242509 20030709
 FDT AU 2003242509 A1 Based on WO 2004008138; EP 1521624 A2 Based on WO
 2004008138; JP 2005532130 W Based on WO 2004008138; AU 2003242509 A8 Based
 on WO 2004008138
 PRAI DK 2002-1091 20020711
 AB WO2004008138 A UPAB: 20040226
 NOVELTY - Extracorporeal adsorption agent (M1), for removing harmful substances responsible of inducing sepsis caused by gram-negative or gram positive bacteria in a mammal, involves treating blood obtained from the mammal by passing the blood through the adsorption column assembly at such a flow rate that a fluidized bed of the particles is formed.
 DETAILED DESCRIPTION - Extracorporeal adsorption (M1), for removing harmful substances responsible of inducing sepsis caused by gram-negative or gram positive bacteria in a mammal, the extracorporeal adsorption being effected by an adsorption column assembly, where the adsorption column assembly comprising a column and an adsorption medium in the form of particles, the sedimented volume of the particles being at the most 80% of the volume of the column, the particles being characterized by carrying an affinity specific molecule with a specific affinity for the LPS portion of the gram-negative bacteria, gram-positive bacteria or harmful substances derived from the gram-positive bacteria, involves treating blood obtained from the mammal by passing the blood through the adsorption column assembly at such a flow rate that a fluidized bed of the particles is formed.
 ACTIVITY - Antibacterial; Immunosuppressive.
 MECHANISM OF ACTION - Removing harmful substances responsible of inducing sepsis.
 The use of extracorporeal adsorption for the treatment of endotoxin-challenged cows was as follows. The cows weighing

500-800 kg were challenged by intravenous injection of 1000 ng lipopolysaccharide (LPS)/kg body weight. After the injection of LPS, the cow was connected to a venous-venous extracorporeal adsorption circuit, comprising a stabilized fluidized bed of polymyxin B-coated particles connected through a switch, the switch being activated by a continuous monitoring device, detecting changes in the serum concentration of haptoglobin in the blood. Clinical parameters, including rectal temperature, heart rate, respiratory frequency, and acute phase protein responses were measured up to one week after the challenge and compared between cows treated by the described extracorporeal method and in untreated cows. Results showed that LPS-challenged cows treated by stand-by extracorporeal adsorption of the animal's blood in a continuous process through a stabilized fluidized bed of polymyxin B-coated particles present with significantly less, significantly less severe and significantly more short-lived clinical signs than comparable, non-treated cows.

USE - (M1) is useful for treating (M2) sepsis caused by gram-negative or gram-positive bacteria in mammal e.g., human being, which involves obtaining blood from the mammal, treating the obtained blood by passing the blood through the adsorption column assembly at such a flow rate that a fluidized bed of the particles is formed, and reinfusing the treated blood into the same mammal. The flow rate of the blood through the column assembly is such that expansion ratio of the fluidized bed is at least 1.3, such as at least 1.5. (M2) further involves injecting the substance into the blood stream of the mammal (claimed).

DESCRIPTION OF DRAWING(S) - The figure shows the principle of continuous extracorporeal adsorption.

Dwg.3/7

TECH

UPTX: 20040226

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Method: In (M1), the treated blood is capable of being reinfused into the same mammal. The adsorption column assembly is adapted for fluidized bed adsorption, in particular stabilized fluidized bed adsorption. The particles have a density of at least 1.3 g/ml and a mean diameter is 5-1000 microns, such as density of at least 1.5 g/ml and a mean diameter is 5-300 microns, preferably a density of at least 1.8 g/ml and a mean diameter in the range of 5-150 microns, and most preferably a density of more than 2.5 g/ml and a mean diameter is 5-75 microns. The affinity specific molecule is chosen from immunoglobulins, peptides, oligonucleotides, receptor proteins, antibiotics and lectins. The affinity specific molecules are chosen from immunoglobulins. Two or more different specific molecules are present on particles within the adsorption medium. The affinity specific molecule is polymyxin B. The affinity specific molecule is chosen from toll-like receptor, most preferably TLR4 or its binding fragments or its multimeric arrangements, CD14, MD2, TLR2 and LBP, and their combinations. The sedimented volume of the particles is at the most 70% of the volume of the column, such as at the most 60% of the volume of the column, e.g. at the most 50% of the volume of the column. The stabilized fluidized bed is placed in line with a switch capable of being activated when a blood substance reaches a pre-set value, the blood substance is monitored by a device, the device is placed in line with the blood circulation, the device sending the activating signal to the switch when the value is reached.

L6 ANSWER 6 OF 6 WPIX COPYRIGHT 2006 THE THOMSON CORP on STN

AN 2004-027278 [03] WPIX

DNN N2004-021623 DNC C2004-009400

TI Transgenic non human animal with no response property to Gram negative bacterial membrane component e.g., lipopolysaccharide, comprises MD-2 gene deficient chromosome which encodes toll-like receptor.

DC B04 D16 P14 S03
 PA (KAGA-N) KAGAKU GIJUTSU SHINKO JIGYODAN
 CYC 1
 PI JP 2003319734 A 20031111 (200403)* 13
 ADT JP 2003319734 A JP 2002-130964 20020502
 PRAI JP 2002-130964 20020502
 AB JP2003319734 A UPAB: 20040112

NOVELTY - Transgenic non-human animal (I) with no response property to Gram negative bacterial membrane component e.g., lipopolysaccharide (LPS), comprises **MD-2** gene deficient chromosome which encodes toll-like receptor 4 (TLR4).

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(1) screening (M1) a of Gram-negative-bacterial membrane component responsive substance, involves introducing a test substance into (I), or introducing a test substance into (I) having **MD-2** gene of different animal; and

(2) diagnosing (M2) the response of different **MD-2** gene in non-human animal, involves transducing **MD-2** gene into (I) and inducing an **endotoxin** shock into (I).

USE - (I) is useful for screening of Gram-negative-bacterial membrane component responsive substance, or for diagnosing the response of different **MD-2** genes in non-human animal (claimed).

(I) is useful for developing a medical agent which is used for further drug development.

ADVANTAGE - (I) enables to screen Gram-negative-bacterial membrane component responsive substance, or to diagnose the response of different **MD-2** gene in non-human animal.

DESCRIPTION OF DRAWING(S) - The figure shows the lipopolysaccharide expression of the macrophage or dendritic cells derived from the **MD-2** genetically engineered mouse. (Drawing includes non-English language text).

Dwg.3/5

TECH UPTX: 20040112

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Transgenic Animal: (I) is a mouse. The plasmid (targeting vector) which comprises a poly A signal and a marker gene, substitutes **MD-2** gene fragment in a mouse. The targeting vector comprises a neomycin resistant gene which is substituted for the first exon of **MD-2** gene and diphtheria **toxin** gene connected with the 3' terminal of the **MD-2** gene. The targeting vector is linearized and transduced into an embryonic stem cell which is deficient in **MD-2** gene. The above embryonic stem cell is injected into the blastocyst of a mouse, thus a chimera mouse is produced. The chimera mouse and the wild-type mouse where crossed to produce a heterozygote. **MD-2** knockout mouse is obtained by intercrossing the heterozygote mouse.

Preferred Method: In (M1), the test substance introduces nucleotide polymorphism of human **MD-2** gene.

L15 ANSWER 1 OF 1 WPIX COPYRIGHT 2006 THE THOMSON CORP on STN
 AN 1992-217016 [26] WPIX
 CR 1989-068849 [09]; 2000-678663 [63]; 2001-637906 [61]
 DNC C1992-098276
 TI Antibacterial fragments of bactericidal-permeability-increasing protein - for treating Gram negative bacterial infections especially in immuno suppressed patients.

DC B04 D16
 IN ELSBACH, P; WEISS, J
 PA (UYNY) UNIV NEW YORK STATE
 CYC 18
 PI WO 9209621 A1 19920611 (199226)* EN 63
 RW: AT BE CH DE DK ES FR GB GR IT LU MC NL SE
 W: AU CA JP
 AU 9191275 A 19920625 (199239)
 EP 563222 A1 19931006 (199340) EN
 R: DE FR GB
 EP 563222 A4 19940608 (199531)
 US 5576292 A 19961119 (199701) 32
 EP 563222 B1 19980225 (199812) EN 31
 R: DE FR GB
 DE 69128968 E 19980402 (199819)
 ADT WO 9209621 A1 WO 1991-US9033 19911203; AU 9191275 A AU 1991-91275
 19911203, WO 1991-US9033 19911203; EP 563222 A1 WO 1991-US9033 19911203,
 EP 1992-902215 19911203; EP 563222 A4 EP 1992-902215 ; US 5576292
 A CIP of US 1987-84335 19870811, CIP of US 1988-228035 19880805, CIP of US
 1990-621473 19901203, Cont of US 1991-754204 19910826, US 1993-173968
 19931223; EP 563222 B1 WO 1991-US9033 19911203, EP 1992-902215 19911203;
 DE 69128968 E DE 1991-628968 19911203, WO 1991-US9033 19911203, EP
 1992-902215 19911203
 FDT AU 9191275 A Based on WO 9209621; EP 563222 A1 Based on WO 9209621; EP
 563222 B1 Based on WO 9209621; DE 69128968 E Based on EP 563222, Based on
 WO 9209621
 PRAI US 1991-754204 19910826; US 1990-621473 19901203;
 US 1987-84335 19870811; US 1988-228035 19880805;
 US 1993-173968 19931223
 AB WO 9209621 A UPAB: 20011217
 Protein (I) comprises specified aminoacid sequence of amino acids 1-199.
 Purified, isolated protein comprises amino acids 1-203; neutralising
 bacterial lipopolysaccharides (LPS) comprises exposing the LPS to a
 neutralising amount of protein (II) comprising N-terminal fragment of
 bactericidal/permeability-increasing protein (BPI); and treating a mammal
 suffering from or at an increased risk of contracting a gram-negative
 bacterial infection comprises administering (II). The bacteria are killed;
 or growth is inhibited, or the LPS is neutralised to prevent or abate
 clinical symptoms associated with the LPS, etc.
 USE/ADVANTAGE - Used to treat mammals with gram-negative bacterial
 infections. The effectiveness of treatment is increased by administering
 the peptides with other bactericidal agents. Peptides will provide
 structural information for the design of future generations of
 antimicrobial agents, and used as probes into the mol. organisation of the
 multifunctional holo-BPI prote
 Dwg.0/10

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(FILE 'CAPLUS' ENTERED AT 11:38:24 ON 03 JUL 2006)

L1	22495	SEA ABB=ON	PLU=ON	TOXIN#/OBI (L) ENDO/OBI OR ENDOTOXIN#/OBI
L2	274	SEA ABB=ON	PLU=ON	MD ?/OBI
L3	26	SEA ABB=ON	PLU=ON	L1 (L) L2
L4	31	SEA ABB=ON	PLU=ON	L1 AND L2
L5	986912	SEA ABB=ON	PLU=ON	CONJUGAT?/OBI OR COMPLEX?/OBI
L6	8	SEA ABB=ON	PLU=ON	L4 AND L5 D SCAN
L7	14	SEA ABB=ON	PLU=ON	((ENDO TOXIN# OR ENDOTOXIN#) (4A) (MD2 OR MD 2))/AB
L8	11	SEA ABB=ON	PLU=ON	L7 NOT L6
L9	62	SEA ABB=ON	PLU=ON	L1 (L) (BOUND/OBI OR BOND?/OBI)
L10	1	SEA ABB=ON	PLU=ON	L9 AND L2 D SCAN
L11	0	SEA ABB=ON	PLU=ON	((ENDO TOXIN# OR ENDOTOXIN#) (S) (BOUND OR BOND?) (S) (MD2 OR MD 2))/AB
L12	20	SEA ABB=ON	PLU=ON	L10 OR L6 OR L8
L13	14	SEA ABB=ON	PLU=ON	L12 AND L1 AND L2
L14	362	SEA ABB=ON	PLU=ON	WEISS J/AU
L15	53	SEA ABB=ON	PLU=ON	GIOANNINI T?/AU
L16	8	SEA ABB=ON	PLU=ON	TEGHANEMT A?/AU
L17	909	SEA ABB=ON	PLU=ON	SUBRAMANIAN R?/AU
L18	1324	SEA ABB=ON	PLU=ON	(L14 OR L15 OR L16 OR L17)
L19	13	SEA ABB=ON	PLU=ON	L18 AND (L1 OR L2)
L20	6	SEA ABB=ON	PLU=ON	L7 AND L18
L21	5	SEA ABB=ON	PLU=ON	L18 AND L1 AND L2
L22	7	SEA ABB=ON	PLU=ON	L20 OR L21

FILE 'MEDLINE' ENTERED AT 12:01:35 ON 03 JUL 2006

L23	410	SEA ABB=ON	PLU=ON	MD2 OR MD 2
L24	152	SEA ABB=ON	PLU=ON	LYMPHOCYTE ANTIGEN 96/CT
L25	425	SEA ABB=ON	PLU=ON	L23 OR L24 E ENDOTOXINS/CT E E3+ALL
L26	18668	SEA ABB=ON	PLU=ON	ENDOTOXINS/CT
L27	23	SEA ABB=ON	PLU=ON	L25 AND L26
L28	941913	SEA ABB=ON	PLU=ON	CONJUGAT? OR BOUND? OR BOND? OR COMPLEX?
L29	15	SEA ABB=ON	PLU=ON	L27 AND L28
L30	30867	SEA ABB=ON	PLU=ON	ENDOTOXIN?
L31	7	SEA ABB=ON	PLU=ON	L25 (S) L28 (S) L30 E WEISS J?/AU
L32	19	SEA ABB=ON	PLU=ON	("WEISS JERROLD"/AU OR "WEISS JERROLD P"/AU) E GIOANNINI T/AU
L33	44	SEA ABB=ON	PLU=ON	("GIOANNINI T"/AU OR "GIOANNINI T L"/AU OR "GIOANNINI THERESA L"/AU OR "GIOANNINI THERESA LEE"/AU) E TEGHANEMT A/AU
L34	7	SEA ABB=ON	PLU=ON	("TEGHANEMT A"/AU OR "TEGHANEMT ATHMANE"/AU) E SUBRAMANIAN R/AU
L35	122	SEA ABB=ON	PLU=ON	("SUBRAMANIAN R"/AU OR "SUBRAMANIAN R B"/AU OR "SUBRAMANIAN R M"/AU OR "SUBRAMANIAN R R"/AU OR "SUBRAMANIAN R SHANKAR"/AU OR "SUBRAMANIAN R V"/AU OR "SUBRAMAN IAN R VENKATA"/AU) E SUBRAMANIAN RAMASWAMY/AU
L36	6	SEA ABB=ON	PLU=ON	"SUBRAMANIAN RAMASWAMY"/AU

AUDET 10/715,876

L37 185 SEA ABB=ON PLU=ON (L32 OR L33 OR L34 OR L35 OR L36)
L38 6 SEA ABB=ON PLU=ON L37 AND L25 AND L30
L39 3 SEA ABB=ON PLU=ON L38 NOT L31

FILE 'BIOSIS' ENTERED AT 12:12:30 ON 03 JUL 2006
L40 534 SEA ABB=ON PLU=ON MD2 OR MD 2
D HIT
L41 32504 SEA ABB=ON PLU=ON ENDO (2W) TOXIN# OR ENDOTOXIN#
L42 21 SEA ABB=ON PLU=ON L41 (S) L40
D HIT
L43 962150 SEA ABB=ON PLU=ON COMPLEX? OR BOUND? OR BOND? OR CONJUGAT?
L44 13 SEA ABB=ON PLU=ON L42 (L) L43
E WEISS J/AU
L45 632 SEA ABB=ON PLU=ON "WEISS J"/AU OR ("WEISS J P"/AU OR "WEISS
J PETER"/AU)
E GIONNINI T/AU
E GIOANNINI T/AU
L46 59 SEA ABB=ON PLU=ON ("GIOANNINI T"/AU OR "GIOANNINI T L"/AU OR
"GIOANNINI THERESA"/AU OR "GIOANNINI THERESA L"/AU OR "GIOANNIN
I THERESA LEE"/AU)
E TEGHANEMT A/AU
E TE GHANEMT A/AU
L47 13 SEA ABB=ON PLU=ON ("TEGHANEMT A"/AU OR "TEGHANEMT ATHMANE"/AU
)
E SUBRAMANIAN R/AU
L48 179 SEA ABB=ON PLU=ON ("SUBRAMANIAN R"/AU OR "SUBRAMANIAN R
B"/AU OR "SUBRAMANIAN R K"/AU OR "SUBRAMANIAN R R"/AU OR
"SUBRAMANIAN R S"/AU OR "SUBRAMANIAN R SHANKAR"/AU OR "SUBRAMAN
IAN R V"/AU)
E SUBRAMANIAN RAMASWAMY/AU
L49 12 SEA ABB=ON PLU=ON "SUBRAMANIAN RAMASWAMY"/AU
L50 878 SEA ABB=ON PLU=ON (L45 OR L46 OR L47 OR L48 OR L49)
L51 5 SEA ABB=ON PLU=ON L50 AND L40 AND L41
L52 1 SEA ABB=ON PLU=ON L51 NOT L44

FILE 'CAPLUS, MEDLINE, BIOSIS' ENTERED AT 12:18:01 ON 03 JUL 2006
L53 22 DUP REM L13 L31 L44 (12 DUPLICATES REMOVED)
ANSWERS '1-14' FROM FILE CAPLUS
ANSWERS '15-17' FROM FILE MEDLINE
ANSWERS '18-22' FROM FILE BIOSIS
L54 7 DUP REM L22 L39 L52 (4 DUPLICATES REMOVED)
ANSWERS '1-7' FROM FILE CAPLUS

=> fil caplus medline biosis
FILE 'CAPLUS' ENTERED AT 12:18:30 ON 03 JUL 2006
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2006 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'MEDLINE' ENTERED AT 12:18:30 ON 03 JUL 2006

FILE 'BIOSIS' ENTERED AT 12:18:30 ON 03 JUL 2006
Copyright (c) 2006 The Thomson Corporation

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L1	22495 SEA FILE=CAPLUS ABB=ON	PLU=ON	TOXIN#/OBI (L) ENDO/OBI OR ENDOTOXIN#/OBI
L2	274 SEA FILE=CAPLUS ABB=ON	PLU=ON	MD 2/OBI
L4	31 SEA FILE=CAPLUS ABB=ON	PLU=ON	L1 AND L2
L5	986912 SEA FILE=CAPLUS ABB=ON	PLU=ON	CONJUGAT?/OBI OR COMPLEX?/OBI
L6	8 SEA FILE=CAPLUS ABB=ON	PLU=ON	L4 AND L5
L7	14 SEA FILE=CAPLUS ABB=ON	PLU=ON	((ENDO TOXIN# OR ENDOTOXIN#) (4A) (MD2 OR MD 2)) /AB
L8	11 SEA FILE=CAPLUS ABB=ON	PLU=ON	L7 NOT L6
L9	62 SEA FILE=CAPLUS ABB=ON	PLU=ON	L1 (L) (BOUND/OBI OR BOND?/OBI)
L10	1 SEA FILE=CAPLUS ABB=ON	PLU=ON	L9 AND L2
L12	20 SEA FILE=CAPLUS ABB=ON	PLU=ON	L10 OR L6 OR L8
L13	14 SEA FILE=CAPLUS ABB=ON	PLU=ON	L12 AND L1 AND L2
L23	410 SEA FILE=MEDLINE ABB=ON	PLU=ON	MD2 OR MD 2
L24	152 SEA FILE=MEDLINE ABB=ON	PLU=ON	LYMPHOCYTE ANTIGEN 96/CT
L25	425 SEA FILE=MEDLINE ABB=ON	PLU=ON	L23 OR L24
L28	941913 SEA FILE=MEDLINE ABB=ON	PLU=ON	CONJUGAT? OR BOUND? OR BOND? OR COMPLEX?
L30	30867 SEA FILE=MEDLINE ABB=ON	PLU=ON	ENDOTOXIN?
L31	7 SEA FILE=MEDLINE ABB=ON	PLU=ON	L25 (S) L28 (S) L30
L40	534 SEA FILE=BIOSIS ABB=ON	PLU=ON	MD2 OR MD 2
L41	32504 SEA FILE=BIOSIS ABB=ON	PLU=ON	ENDO (2W) TOXIN# OR ENDOTOXIN#
L42	21 SEA FILE=BIOSIS ABB=ON	PLU=ON	L41 (S) L40
L43	962150 SEA FILE=BIOSIS ABB=ON	PLU=ON	COMPLEX? OR BOUND? OR BOND? OR CONJUGAT?
L44	13 SEA FILE=BIOSIS ABB=ON	PLU=ON	L42 (L) L43
L53	22 DUP REM L13 L31 L44	(12 DUPLICATES REMOVED)	

=> d que 154

L1	22495 SEA FILE=CAPLUS ABB=ON	PLU=ON	TOXIN#/OBI (L) ENDO/OBI OR ENDOTOXIN#/OBI
L2	274 SEA FILE=CAPLUS ABB=ON	PLU=ON	MD 2/OBI
L7	14 SEA FILE=CAPLUS ABB=ON	PLU=ON	((ENDO TOXIN# OR ENDOTOXIN#) (4A) (MD2 OR MD 2)) /AB
L14	362 SEA FILE=CAPLUS ABB=ON	PLU=ON	WEISS J/AU
L15	53 SEA FILE=CAPLUS ABB=ON	PLU=ON	GIOANNINI T?/AU
L16	8 SEA FILE=CAPLUS ABB=ON	PLU=ON	TEGHANEMT A?/AU
L17	909 SEA FILE=CAPLUS ABB=ON	PLU=ON	SUBRAMANIAN R?/AU
L18	1324 SEA FILE=CAPLUS ABB=ON	PLU=ON	(L14 OR L15 OR L16 OR L17)
L20	6 SEA FILE=CAPLUS ABB=ON	PLU=ON	L7 AND L18
L21	5 SEA FILE=CAPLUS ABB=ON	PLU=ON	L18 AND L1 AND L2
L22	7 SEA FILE=CAPLUS ABB=ON	PLU=ON	L20 OR L21
L23	410 SEA FILE=MEDLINE ABB=ON	PLU=ON	MD2 OR MD 2
L24	152 SEA FILE=MEDLINE ABB=ON	PLU=ON	LYMPHOCYTE ANTIGEN 96/CT

L25	425 SEA FILE=MEDLINE ABB=ON	PLU=ON	L23 OR L24
L28	941913 SEA FILE=MEDLINE ABB=ON	PLU=ON	CONJUGAT? OR BOUND? OR BOND?
	OR COMPLEX?		
L30	30867 SEA FILE=MEDLINE ABB=ON	PLU=ON	ENDOTOXIN?
L31	7 SEA FILE=MEDLINE ABB=ON	PLU=ON	L25 (S) L28 (S) L30
L32	19 SEA FILE=MEDLINE ABB=ON	PLU=ON	("WEISS JERROLD"/AU OR "WEISS JERROLD P"/AU)
L33	44 SEA FILE=MEDLINE ABB=ON	PLU=ON	("GIOANNINI T"/AU OR "GIOANNINI T L"/AU OR "GIOANNINI THERESA L"/AU OR "GIOANNINI THERESA LEE"/AU)
L34	7 SEA FILE=MEDLINE ABB=ON	PLU=ON	("TEGHANEMT A"/AU OR "TEGHANEMT ATHMANE"/AU)
L35	122 SEA FILE=MEDLINE ABB=ON	PLU=ON	("SUBRAMANIAN R"/AU OR "SUBRAMANIAN R B"/AU OR "SUBRAMANIAN R M"/AU OR "SUBRAMANIAN R R"/AU OR "SUBRAMANIAN R SHANKAR"/AU OR "SUBRAMANIAN R V"/AU OR "SUBRAMANIAN R VENKATA"/AU)
L36	6 SEA FILE=MEDLINE ABB=ON	PLU=ON	"SUBRAMANIAN RAMASWAMY"/AU
L37	185 SEA FILE=MEDLINE ABB=ON	PLU=ON	(L32 OR L33 OR L34 OR L35 OR L36)
L38	6 SEA FILE=MEDLINE ABB=ON	PLU=ON	L37 AND L25 AND L30
L39	3 SEA FILE=MEDLINE ABB=ON	PLU=ON	L38 NOT L31
L40	534 SEA FILE=BIOSIS ABB=ON	PLU=ON	MD2 OR MD 2
L41	32504 SEA FILE=BIOSIS ABB=ON	PLU=ON	ENDO (2W) TOXIN# OR ENDOTOXIN#
L42	21 SEA FILE=BIOSIS ABB=ON	PLU=ON	L41 (S) L40
L43	962150 SEA FILE=BIOSIS ABB=ON	PLU=ON	COMPLEX? OR BOUND? OR BOND? OR CONJUGAT?
L44	13 SEA FILE=BIOSIS ABB=ON	PLU=ON	L42 (L) L43
L45	632 SEA FILE=BIOSIS ABB=ON	PLU=ON	"WEISS J"/AU OR ("WEISS J PETER"/AU)
L46	59 SEA FILE=BIOSIS ABB=ON	PLU=ON	("GIOANNINI T"/AU OR "GIOANNINI T L"/AU OR "GIOANNINI THERESA"/AU OR "GIOANNINI THERESA L"/AU OR "GIOANNINI THERESA LEE"/AU)
L47	13 SEA FILE=BIOSIS ABB=ON	PLU=ON	("TEGHANEMT A"/AU OR "TEGHANEMT ATHMANE"/AU)
L48	179 SEA FILE=BIOSIS ABB=ON	PLU=ON	("SUBRAMANIAN R"/AU OR "SUBRAMANIAN R B"/AU OR "SUBRAMANIAN R K"/AU OR "SUBRAMANIAN R R"/AU OR "SUBRAMANIAN R S"/AU OR "SUBRAMANIAN R SHANKAR"/AU OR "SUBRAMANIAN R V"/AU)
L49	12 SEA FILE=BIOSIS ABB=ON	PLU=ON	"SUBRAMANIAN RAMASWAMY"/AU
L50	878 SEA FILE=BIOSIS ABB=ON	PLU=ON	(L45 OR L46 OR L47 OR L48 OR L49)
L51	5 SEA FILE=BIOSIS ABB=ON	PLU=ON	L50 AND L40 AND L41
L52	1 SEA FILE=BIOSIS ABB=ON	PLU=ON	L51 NOT L44
L54	7 DUP REM L22 L39 L52	(4 DUPLICATES REMOVED)	

=> d .ca l53 1-14;d ibib ab ct l53 15-22;d ibib ab ct l54 1-7

L53 ANSWER 1 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 1
 ACCESSION NUMBER: 2005:1190853 CAPLUS
 DOCUMENT NUMBER: 144:86458
 TITLE: Pharmacological Inhibition of Endotoxin
 Responses Is Achieved by Targeting the TLR4
 Coreceptor, MD-2
 AUTHOR(S): Visintin, Alberto; Halmen, Kristen A.; Latz, Eicke;
 Monks, Brian G.; Golenbock, Douglas T.
 CORPORATE SOURCE: Division of Infectious Diseases and Immunology,
 University of Massachusetts Medical School, Worcester,
 MA, 01655, USA

SOURCE: Journal of Immunology (2005), 175(10), 6465-6472
 CODEN: JOIMA3; ISSN: 0022-1767

PUBLISHER: American Association of Immunologists

DOCUMENT TYPE: Journal

LANGUAGE: English

ED Entered STN: 09 Nov 2005

AB The detection of Gram-neg. LPS depends upon the proper function of the TLR4-MD-2 receptor complex in immune cells. TLR4 is the signal transduction component of the LPS receptor, whereas **MD-2** is the **endotoxin**-binding unit. MD-2 appears to activate TLR4 when bound to TLR4 and ligated by LPS. Only the monomeric form of MD-2 was found to bind LPS and only monomeric MD-2 interacts with TLR4. Monomeric MD-2 binds TLR4 with an apparent Kd of 12 nM; this binding avidity was unaltered in the presence of endotoxin. E5564, an LPS antagonist, appears to inhibit cellular activation by competitively preventing the binding of LPS to MD-2. Depletion of endogenous soluble MD-2 from human serum, with an immobilized TLR4 fusion protein, abrogated TLR4-mediated LPS responses. By determining the concentration of added-back MD-2 that restored normal LPS responsiveness, the concentration of MD-2 was estimated to be .apprx.50 nM. Similarly, purified TLR4-Fc fusion protein, when added to the supernatants of TLR4-expressing cells in culture, inhibited the interaction of MD-2 with TLR4, thus preventing LPS stimulation. The ability to inhibit the effects of LPS as a result of the binding of TLR4-Fc or E5564 to MD-2 highlights MD-2 as the logical target for drug therapies designed to pharmacol. intervene against endotoxin-induced disease.

CC 15-10 (Immunochemistry)
 Section cross-reference(s): 1, 13

ST **endotoxin** interaction TLR4 receptor MD2 protein

IT Drug targets
 (MD-2 protein)

IT Proteins
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (MD-2; monomeric MD-2 interacts with **endotoxin** or TLR4 receptor)

IT Receptors
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (TLR-4 (Toll-like receptor-4); monomeric MD-2 interacts with **endotoxin** or TLR4 receptor)

IT Lipopolysaccharides
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (bacterial; monomeric MD-2 interacts with **endotoxin** or TLR4 receptor)

IT Toxins
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (**endotoxins**; monomeric MD-2 interacts with **endotoxin** or TLR4 receptor)

IT Blood
 (indexes; for MD-2 protein)

IT Human
 (monomeric MD-2 interacts with **endotoxin** or TLR4 receptor)

IT Sepsis
 (monomeric MD-2 interacts with **endotoxin** or TLR4 receptor in relation to)

IT Stoichiometry
 (of monomeric MD-2 interaction with TLR4 receptor)

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L53 ANSWER 2 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 2
 ACCESSION NUMBER: 2005:1016771 CAPLUS
 DOCUMENT NUMBER: 143:304613
 TITLE: Molecular Basis of Reduced Potency of Underacylated Endotoxins
 AUTHOR(S): Teghanemt, Athmane; Zhang, DeSheng; Levis, Erika N.; Weiss, Jerrold P.; Gioannini, Theresa L.
 CORPORATE SOURCE: Inflammation Program, Department of Internal Medicine, Coralville, IA, 52241, USA
 SOURCE: Journal of Immunology (2005), 175(7), 4669-4676
 CODEN: JOIMA3; ISSN: 0022-1767
 PUBLISHER: American Association of Immunologists
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 ED Entered STN: 21 Sep 2005
 AB Potent TLR4-dependent cell activation by Gram-neg. bacterial endotoxins depends on sequential endotoxin-protein and protein-protein interactions with LPS-binding protein, CD14, myeloid differentiation protein 2 (MD-2), and TLR4. Previous studies have suggested that reduced agonist potency of underacylated endotoxins (i.e., tetra- or penta- vs. hexa-acylated) is determined by post-CD14 interactions. To better define the mol. basis of the differences in agonist potency of endotoxins differing in fatty acid acylation, the authors compared endotoxins (lipooligosaccharides (LOS)) from hexa-acylated wild-type (wt), penta-acylated mutant msbB meningococcal strains as well as tetra-acylated LOS generated by treatment of wt LOS with the deacylating enzyme, acyloxyacylhydrolase. To facilitate assay of endotoxin:protein and endotoxin:cell interactions, the endotoxins were purified after metabolic labeling with [3H]- or [14C]acetate. All LOS species tested formed monomeric complexes with MD-2 in an LPS-binding protein- and CD14-dependent manner with similar efficiency. However, msbB LOS:MD-2 and acyloxyacylhydrolase-treated LOS:MD-2 were at least 10-fold less potent in inducing TLR4-dependent cell activation than wt LOS:MD-2 and partially antagonized the action of wt LOS:MD-2. These findings suggest that underacylated endotoxins produce decreased TLR4-dependent cell activation by altering the interaction of the **endotoxin:MD-2** complex with TLR4 in a way that reduces receptor activation. Differences in potency among these endotoxin species is determined not by different aggregate properties, but by different properties of monomeric **endotoxin:MD-2** complexes.
 CC 15-10 (Immunochemistry)
 Section cross-reference(s): 4, 10
 ST acylation **endotoxin** TLR4 receptor activation
 IT Proteins
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (MD-2; structural features of **endotoxins**
 inducing TLR4 receptor-dependent cell activation)
 IT Receptors
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (TLR-4 (Toll-like receptor-4); structural features of
 endotoxins inducing TLR4 receptor-dependent cell activation)
 IT Toxins
 RL: BSU (Biological study, unclassified); PRP (Properties); BIOL
 (Biological study)
 (**endotoxins**; structural features of **endotoxins**
 inducing TLR4 receptor-dependent cell activation)
 IT Lipopolysaccharides
 RL: BSU (Biological study, unclassified); PRP (Properties); BIOL

(Biological study)

(lipooligosaccharides; structural features of **endotoxins**
inducing TLR4 receptor-dependent cell activation)IT Structure-activity relationship
(of acylation variants of **endotoxins**)IT Cell activation
(structural features of **endotoxins** inducing TLR4
receptor-dependent cell activation)IT CD14 (antigen)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(structural features of **endotoxins** inducing TLR4
receptor-dependent cell activation)IT 140936-27-2 864814-65-3 864814-66-4
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL
(Biological study)
(structural features of **endotoxins** inducing TLR4
receptor-dependent cell activation)REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L53 ANSWER 3 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 4

ACCESSION NUMBER: 2005:489362 CAPLUS

DOCUMENT NUMBER: 143:95745

TITLE: Monomeric **endotoxin:protein**
complexes are essential for TLR4-dependent
cell activationAUTHOR(S): Gioannini, T. L.; Teghanemt, A.; Zhang, De S.; Levis,
E. N.; Weiss, J. P.CORPORATE SOURCE: Department of Internal Medicine, Roy J. and Lucille A.
Carver College of Medicine, University of Iowa and the
Veterans' Administration Medical Center, Iowa City,
IA, USASOURCE: Journal of Endotoxin Research (2005), 11(2), 117-123
CODEN: JENREB; ISSN: 0968-0519

PUBLISHER: Maney Publishing

DOCUMENT TYPE: Journal

LANGUAGE: English

ED Entered STN: 09 Jun 2005

AB Potent TLR4-dependent cell activation by Gram-neg. bacterial endotoxin
depends on sequential endotoxin-protein and protein-protein interactions
with LBP, CD14, MD-2 and TLR4. LBP and CD14 combine, in an
albumin-dependent fashion, to extract single endotoxin mols. from purified
endotoxin aggregates (Eagg) or the bacterial outer membrane and form
monomeric endotoxin:CD14 complexes that are the preferred presentation of
endotoxin for transfer to MD-2. Endotoxin in endotoxin:CD14 is readily
transferred to MD-2, again in an albumin-dependent manner, to form
monomeric endotoxin:MD-2 complex. This monomeric endotoxin:protein
complex (endotoxin:MD-2) activates TLR4 at picomolar concns.,
independently of albumin, and is, therefore, the apparent ligand in
endotoxin-dependent TLR4 activation. Tetra-, penta-, and hexa-acylated
forms of meningococcal endotoxin (LOS) react similarly with LBP, CD14, and
MD-2 to form endotoxin:MD-2 complexes. However, tetra- and penta-acylated
LOS:MD-2 complexes are less potent TLR4 agonists than hexa-acylated
LOS:MD-2. This is mirrored in the reduced activity of tetra-, penta- vs.
hexa-acylated LOS aggregates (LOSagg) + LBP toward cells containing mCD14,
MD-2, and TLR4. Therefore, changes in agonist potency of under-acylated
meningococcal LOS are determined by differences in properties of monomeric
endotoxin:MD-2.

CC 15-10 (Immunochemistry)

ST **endotoxin protein complex TLR4 receptor cell**

activation
IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(MD-2; monomeric **endotoxin:protein complexes** are essential for TLR4 receptor-dependent cell activation)

IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4); monomeric **endotoxin:protein complexes** are essential for TLR4 receptor-dependent cell activation)

IT Blood vessel
(endothelium; monomeric **endotoxin:protein complexes** are essential for TLR4 receptor-dependent cell activation)

IT Toxins
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(endotoxins; monomeric **endotoxin:protein complexes** are essential for TLR4 receptor-dependent cell activation)

IT Glycolipids
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(lipoooligosaccharides; monomeric **endotoxin:protein complexes** are essential for TLR4 receptor-dependent cell activation)

IT Cell activation
(monomeric **endotoxin:protein complexes** are essential for TLR4 receptor-dependent cell activation)

IT CD14 (antigen)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(monomeric **endotoxin:protein complexes** are essential for TLR4 receptor-dependent cell activation)

IT Albumins, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(serum; monomeric **endotoxin:protein complexes** are essential for TLR4 receptor-dependent cell activation)

IT Endothelium
(vascular; monomeric **endotoxin:protein complexes** are essential for TLR4 receptor-dependent cell activation)

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L53 ANSWER 4 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 6
ACCESSION NUMBER: 2004:292853 CAPLUS
DOCUMENT NUMBER: 140:401625
TITLE: Isolation of an **endotoxin-MD-2 complex** that produces Toll-like receptor 4-dependent cell activation at picomolar concentrations
AUTHOR(S): Gioannini, Theresa L.; Teghanemt, Athmane; Zhang, DeSheng; Coussens, Nathan P.; Dockstader, Wendie; Ramaswamy, S.; Weiss, Jerrold P.
CORPORATE SOURCE: Inflammation Program, Department of Internal Medicine, and Department of Biochemistry Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Veterans Affairs Medical Center, Iowa City, IA, 52242, USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (2004), 101(12), 4186-4191

CODEN: PNASA6; ISSN: 0027-8424

PUBLISHER: National Academy of Sciences

DOCUMENT TYPE: Journal

LANGUAGE: English

ED Entered STN: 09 Apr 2004

AB Host proinflammatory responses to minute amounts of endotoxins derived from many Gram-neg. bacteria require the interaction of lipopolysaccharide-binding protein (LBP), CD14, Toll-like receptor 4 (TLR4) and MD-2. Optimal sensitivity to endotoxin requires an ordered series of endotoxin-protein and protein-protein interactions. At substoichiometric concns., LBP facilitates delivery of endotoxin aggregates to soluble CD14 (sCD14) to form monomeric endotoxin-sCD14 complexes. Subsequent interactions of endotoxin-sCD14 with TLR4 and/or MD-2 have not been specifically defined. This study reports the purification of a stable, monomeric, bioactive endotoxin-MD-2 complex generated by treatment of endotoxin-sCD14 with recombinant MD-2. Efficient generation of this complex occurred at picomolar concns. of endotoxin and nanogram per mL doses of MD-2 and required presentation of endotoxin to MD-2 as a monomeric endotoxin-CD14 complex. TLR4-dependent delivery of endotoxin to human embryonic kidney (HEK) cells and cell activation at picomolar concns. of endotoxin occurred with the purified endotoxin-MD-2 complex, but not with purified endotoxin aggregates with or without LBP and/or sCD14. The presence of excess MD-2 inhibited delivery of endotoxin-MD-2 to HEK/TLR4 cells and cell activation. These findings demonstrate that TLR4-dependent activation of host cells by picomolar concns. of endotoxin occurs by sequential interaction and transfer of endotoxin to LBP, CD14, and MD-2 and simultaneous engagement of endotoxin and TLR4 by MD-2.

CC 4-5 (Toxicology)

ST endotoxin MD2 complex CD14 TLR4 LBP

IT Animal cell line

(HEK; isolation of an **endotoxin-MD-2 complex** that produces Toll-like receptor 4-dependent cell activation at picomolar concns. in HEK cells)

IT Proteins

RL: BSU (Biological study, unclassified); BIOL (Biological study) (LPS-LBP (lipopolysaccharide-containing lipopolysaccharide-binding protein); isolation of an **endotoxin-MD-2 complex** that produces Toll-like receptor 4-dependent cell activation at picomolar concns. in HEK cells)

IT Proteins

RL: BSU (Biological study, unclassified); BIOL (Biological study) (**MD-2, complexes with endotoxin**; isolation of an **endotoxin-MD-2 complex** that produces Toll-like receptor 4-dependent cell activation at picomolar concns. in HEK cells)

IT Receptors

RL: BSU (Biological study, unclassified); BIOL (Biological study) (TLR-4 (Toll-like receptor-4); isolation of an **endotoxin-MD-2 complex** that produces Toll-like receptor 4-dependent cell activation at picomolar concns. in HEK cells)

IT CD14 (antigen)

RL: BSU (Biological study, unclassified); BIOL (Biological study) (**complexes with endotoxin**; isolation of an **endotoxin-MD-2 complex** that produces Toll-like receptor 4-dependent cell activation at picomolar concns. in HEK cells)

IT Toxins

RL: ADV (Adverse effect, including toxicity); BSU (Biological study, unclassified); BIOL (Biological study) (**endotoxins, complexes with CD14 and MD-**

2; isolation of an **endotoxin-MD-2 complex** that produces Toll-like receptor 4-dependent cell activation at picomolar concns. in HEK cells)

IT Human

(isolation of an **endotoxin-MD-2 complex** that produces Toll-like receptor 4-dependent cell activation at picomolar concns. in HEK cells)

REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L53 ANSWER 5 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 8

ACCESSION NUMBER: 2004:1074780 CAPLUS

DOCUMENT NUMBER: 142:238068

TITLE: **Endotoxin recognition and signal transduction by the TLR4/MD2-complex**

AUTHOR(S): Fitzgerald, Katherine A.; Rowe, Daniel C.; Golenbock, Douglas T.

CORPORATE SOURCE: Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, MA, 01605, USA

SOURCE: Microbes and Infection (2004), 6(15), 1361-1367
CODEN: MCINFS; ISSN: 1286-4579

PUBLISHER: Elsevier B.V.

DOCUMENT TYPE: Journal; General Review

LANGUAGE: English

ED Entered STN: 16 Dec 2004

AB A review. Bacterial lipopolysaccharides are recognized in mammals by a receptor complex composed of CD14, Toll-like receptor (TLR)-4, and MD-2. Transduction of signaling is achieved following the recruitment of a combination of four Toll-interleukin-1 resistance (TIR)-domain-containing adapter mols., which provide a structural platform enabling the recruitment and activation of downstream effectors essential for pathway-specific transcription factor activation and inflammatory gene expression.

CC 15-0 (Immunochemistry)

Section cross-reference(s): 14

ST review **endotoxin** lipopolysaccharide recognition signaling TLR4

MD2 complex

IT Proteins

RL: BSU (Biological study, unclassified); BIOL (Biological study)
(MD-2; bacterial lipopolysaccharide/
endotoxin recognition and signal transduction by TLR4/MD2 complex)

IT Receptors

RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4); bacterial lipopolysaccharide/
endotoxin recognition and signal transduction by TLR4/MD2 complex)

IT CD14 (antigen)

RL: BSU (Biological study, unclassified); BIOL (Biological study)
(bacterial lipopolysaccharide/**endotoxin** recognition and
signal transduction by CD14/TLR4/MD2 complex)

IT Inflammation

Signal transduction, biological
(bacterial lipopolysaccharide/**endotoxin** recognition and
signal transduction by TLR4/MD2 complex)

IT Lipopolysaccharides

RL: BSU (Biological study, unclassified); BIOL (Biological study)
(bacterial; bacterial lipopolysaccharide/**endotoxin**
recognition and signal transduction by TLR4/MD2 complex)

REFERENCE COUNT: 53 THERE ARE 53 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L53 ANSWER 6 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN
 ACCESSION NUMBER: 2005:451419 CAPLUS
 DOCUMENT NUMBER: 142:476294
 TITLE: **Endotoxin shock medicine for TLR4-MD-2 composite as target**
 INVENTOR(S): Miyake, Kensuke; Takamura, Sachiko
 PATENT ASSIGNEE(S): Japan Science and Technology Agency, Japan
 SOURCE: PCT Int. Appl., 30 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: Japanese
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2005047330	A1	20050526	WO 2004-JP14194	20040928
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				

PRIORITY APPLN. INFO.: JP 2003-387173 A 20031117
 ED Entered STN: 27 May 2005
 AB There are provided an anti-TLR4-MD-2 monoclonal antibody capable of specifically recognizing TLR4-MD-2 composite; an **endotoxin** shock medicine for TLR4-MD-2 composite as target, comprising the anti-TLR4-MD-2 monoclonal antibody; and a method of treating endotoxin shock medicine with the use of the anti-TLR4-MD-2 monoclonal antibody against TLR4-MD-2 composite as target. The anti-TLR4-MD-2 monoclonal antibody having an effect of suppressing endotoxin shock is produced by increasing the production of TNF against endotoxin shock, while not exerting an effect of B-cell proliferation inhibition and an effect of TNF production inhibition in macrophages, through *in vitro* LPS stimulation.
 IC ICM C07K016-28
 ICS A61K039-395; A61P039-02; G01N033-15; G01N033-50; C12P021-08
 CC 1-12 (Pharmacology)
 ST **endotoxin** shock monoclonal antibody anti tlrmd2
 IT Cell proliferation
 (B cell; **endotoxin** shock medicine for TLR4-MD-2 composite as target)
 IT Receptors
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (TLR-4 (Toll-like receptor-4); **endotoxin** shock medicine for TLR4-MD-2 composite as target)
 IT Macrophage
 (**endotoxin** shock medicine for TLR4-MD-2 composite as target)
 IT Tumor necrosis factors
 RL: BSU (Biological study, unclassified); BIOL (Biological study)

(endotoxin shock medicine for TLR4-MD-2 composite as target)

- IT Toxins
 RL: ADV (Adverse effect, including toxicity); BIOL (Biological study)
 (endotoxins; endotoxin shock medicine for TLR4-MD-2 composite as target)
- IT Antibodies and Immunoglobulins
 RL: PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
 (monoclonal, anti-TLR4-MD-2; endotoxin shock medicine for TLR4-MD-2 composite as target)
- IT B cell (lymphocyte)
 (proliferation; endotoxin shock medicine for TLR4-MD-2 composite as target)
- IT Shock (circulatory collapse)
 (septic; endotoxin shock medicine for TLR4-MD-2 composite as target)

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L53 ANSWER 7 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2005:426231 CAPLUS

DOCUMENT NUMBER: 142:480799

TITLE: Preparation of complexes of endotoxin and MD-2 and uses thereof to modulate TLR4 receptor-dependent cell activation by endotoxin

INVENTOR(S): Weiss, Jerrold P.; Gioannini, Theresa L.; Teghanemt, Athamane; Subramanian, Ramaswamy

PATENT ASSIGNEE(S): USA

SOURCE: U.S. Pat. Appl. Publ., 34 pp.

CODEN: USXXCO

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2005106179	A1	20050519	US 2003-715876	20031117
WO 2005049067	A1	20050602	WO 2004-US38375	20041117
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				

PRIORITY APPLN. INFO.: US 2003-715876 A 20031117

ED Entered STN: 19 May 2005

AB The disclosed invention provides purified water soluble complexes of endotoxin and MD-2. The invention also provides a method for making the complexes of the invention and a method for isolating complexes of the invention. Also provided are the method of using the complexes of the invention, e.g. method to increase or inhibit TLR4 receptor-dependent activation of cells by endotoxin in vitro or in vivo. Methods using

complexes with mutant endotoxin are useful to decrease undesirable endotoxin-mediated inflammation. Methods using complexes with wild-type endotoxin are of use in promoting innate immunity and as immune adjuvants. The results of one example demonstrate that in primary cultures of human airway epithelia TLR4, but little or no MD-2 is expressed, so the cells are relatively unresponsive to added endotoxin. However, the cell responsiveness to endotoxin is markedly amplified by either the endogenous expression or exogenous addition of MD-2.

- IC ICM A61K039-02
 ICS C07K014-195
 INCL 424235100; 530395000
 CC 15-10 (Immunochemistry)
 ST **endotoxin MD2 complex** TLR4 receptor cell activation
 IT CD14 (antigen)
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (CD14 requirement in preparation of **complexes** of bacterial
 endotoxin and **MD-2**)
 IT Animal cell line
 (Hek 293; preparation of **complexes** of bacterial **endotoxin**
 and **MD-2** and uses thereof to modulate TLR4
 receptor-dependent cell activation by **endotoxin**)
 IT Proteins
 RL: BPN (Biosynthetic preparation); BUU (Biological use, unclassified);
 BIOL (Biological study); PREP (Preparation); USES (Uses)
 (**MD-2, endotoxin complexes**;
 preparation of **complexes** of bacterial **endotoxin** and
 MD-2 and uses thereof to modulate TLR4
 receptor-dependent cell activation by **endotoxin**)
 IT Receptors
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (TLR-4 (Toll-like receptor-4); preparation of **complexes** of
 bacterial **endotoxin** and **MD-2** and uses
 thereof to modulate TLR4 receptor-dependent cell activation by
 endotoxin)
 IT Immunostimulants
 (adjuvants; preparation of **complexes** of bacterial
 endotoxin and **MD-2** and uses thereof to
 modulate TLR4 receptor-dependent cell activation by **endotoxin**
 in)
 IT Glycolipids
 RL: BPN (Biosynthetic preparation); BUU (Biological use, unclassified);
 BIOL (Biological study); PREP (Preparation); USES (Uses)
 (bacterial; preparation of **complexes** of bacterial
 endotoxin and **MD-2** and uses thereof to
 modulate TLR4 receptor-dependent cell activation by **endotoxin**
)
 IT Drug delivery systems
 (carriers; preparation of **complexes** of bacterial **endotoxin**
 and **MD-2** and uses thereof to modulate TLR4
 receptor-dependent cell activation by **endotoxin**)
 IT Toxins
 RL: BPN (Biosynthetic preparation); BUU (Biological use, unclassified);
 BIOL (Biological study); PREP (Preparation); USES (Uses)
 (**endotoxins, MD-2 complexes**;
 preparation of **complexes** of bacterial **endotoxin** and
 MD-2 and uses thereof to modulate TLR4
 receptor-dependent cell activation by **endotoxin**)
 IT Toxins
 RL: BPN (Biosynthetic preparation); BUU (Biological use, unclassified);
 BIOL (Biological study); PREP (Preparation); USES (Uses)

(**endotoxins**, acylated; preparation of **complexes** of bacterial **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**)

IT Respiratory system
(epithelium; preparation of **complexes** of bacterial **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin** in)

IT Immunity
(innate; preparation of **complexes** of bacterial **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin** in)

IT Cell activation
Escherichia
Escherichia coli
Francisella
Francisella tularensis
Haemophilus
Haemophilus influenzae
Neisseria
Neisseria meningitidis
Pseudomonas
Pseudomonas aeruginosa
Salmonella
Salmonella typhimurium
(preparation of **complexes** of bacterial **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**)

IT Anti-inflammatory agents
Human
(preparation of **complexes** of bacterial **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin** in)

IT Epithelium
(respiratory tract; preparation of **complexes** of bacterial **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin** in)

IT 852008-84-5 852009-59-7 852009-60-0 852009-61-1 852009-62-2
852009-63-3 852009-64-4 852009-65-5 852009-66-6 852009-67-7
852009-68-8 852009-69-9 852009-70-2 852009-71-3 852009-72-4
852009-73-5

RL: PRP (Properties)
(unclaimed nucleotide sequence; preparation of **complexes** of **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**)

L53 ANSWER 8 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2004:1089125 CAPLUS

DOCUMENT NUMBER: 142:175247

TITLE: Protein-bound polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-binding protein and CD14 functions

AUTHOR(S): Asai, Yasuyuki; Takaori, Kyoichi; Yamamoto, Tsuyoshi; Ogawa, Tomohiko

CORPORATE SOURCE: Department of Oral Microbiology, Asahi University
School of Dentistry, Mizuho, Gifu, 501-0296, Japan

SOURCE: FEMS Immunology and Medical Microbiology (2005),
 43(1), 91-98
 CODEN: FIMIEV; ISSN: 0928-8244

PUBLISHER: Elsevier B.V.

DOCUMENT TYPE: Journal

LANGUAGE: English

ED Entered STN: 20 Dec 2004

AB The protein-bound polysaccharide isolated from basidiomycetes (PSK) is a biol. response modifier capable of exhibiting various biol. activities, such as antitumor and antimicrobial effects. In the present study, the authors found that PSK suppressed interleukin (IL)-6 production in murine peritoneal macrophages stimulated with endotoxic lipopolysaccharide (LPS) and its synthetic lipid A (compound 506). Nitric oxide production and p38 mitogen-associated protein kinase phosphorylation induced in a murine macrophage cell line, J774-A1, by LPS and compound 506 were also inhibited by PSK. Further, PSK distinctly suppressed nuclear factor- κ B activation in Ba/F3 cells expressing mouse Toll-like receptor 4 and MD-2, following stimulation with LPS and compound 506, however, not with Taxol. These PSK-induced inhibitory activities were caused by inhibition of the phys. assocns. of LPS with LPS-binding protein (LBP) and CD14. PSK also protected mice from LPS-induced lethality, presumably by down-regulating IL-6 and tumor necrosis factor- α concns. in serum. These findings indicate that PSK, which also has an ability to regulate LBP/CD14 functions, may be useful for clin. control of endotoxic sepsis.

CC 15-10 (Immunochemistry)

IT Proteins

RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (MD-2; protein-bound polysaccharide
 isolated from basidiomycetes inhibits endotoxin-induced
 activation by blocking lipopolysaccharide-binding protein and CD14
 functions)

IT Transcription factors

RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (NF- κ B (nuclear factor of κ light chain gene enhancer in
 B-cells); protein-bound polysaccharide isolated from
 basidiomycetes inhibits endotoxin-induced activation by
 blocking lipopolysaccharide-binding protein and CD14 functions)

IT Receptors

RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (TLR-4 (Toll-like receptor-4); protein-bound polysaccharide
 isolated from basidiomycetes inhibits endotoxin-induced
 activation by blocking lipopolysaccharide-binding protein and CD14
 functions)

IT Proteins

RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (lipopolysaccharide-binding; protein-bound polysaccharide
 isolated from basidiomycetes inhibits endotoxin-induced
 activation by blocking lipopolysaccharide-binding protein and CD14
 functions)

IT Peritoneum

(macrophage; protein-bound polysaccharide isolated from
 basidiomycetes inhibits endotoxin-induced activation by
 blocking lipopolysaccharide-binding protein and CD14 functions)

IT Macrophage

(peritoneal; protein-bound polysaccharide isolated from
 basidiomycetes inhibits endotoxin-induced activation by
 blocking lipopolysaccharide-binding protein and CD14 functions)

IT Basidiomycota

(protein-bound polysaccharide isolated from basidiomycetes
 inhibits endotoxin-induced activation by blocking

IT lipopolysaccharide-binding protein and CD14 functions)
 IT CD14 (antigen)
 Interleukin 6
 Tumor necrosis factors
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (protein-bound polysaccharide isolated from basidiomycetes
 inhibits **endotoxin**-induced activation by blocking
 lipopolysaccharide-binding protein and CD14 functions)
 IT Polysaccharides, biological studies
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (protein-bound; protein-bound polysaccharide
 isolated from basidiomycetes inhibits **endotoxin**-induced
 activation by blocking lipopolysaccharide-binding protein and CD14
 functions)
 IT 10102-43-9, Nitric oxide, biological studies 165245-96-5, p38 MAP kinase
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (protein-bound polysaccharide isolated from basidiomycetes
 inhibits **endotoxin**-induced activation by blocking
 lipopolysaccharide-binding protein and CD14 functions)
 REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS
 RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L53 ANSWER 9 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN
 ACCESSION NUMBER: 2005:70134 CAPLUS
 DOCUMENT NUMBER: 142:152686
 TITLE: **Endotoxin** shock and TLR
 AUTHOR(S): Takamura, Sachiko Akashi
 CORPORATE SOURCE: Inst. Med. Sci., The Univ. Tokyo, Japan
 SOURCE: Ensho to Men'eki (2004), Volume Date 2005, 13(1),
 37-44
 CODEN: ENMEFA; ISSN: 0918-8371
 PUBLISHER: Sentan Igakusha
 DOCUMENT TYPE: Journal; General Review
 LANGUAGE: Japanese
 ED Entered STN: 27 Jan 2005
 AB A review on roles of Toll-like receptor (TLR) in endotoxin shock,
 discussing recognition mechanism of **endotoxin**
 (lipopolysaccharide : LPS) by TLR4/MD-2 complex,
 essential role of MD-2 in LPS responsiveness and
endotoxin shock, MD proteins regulating LPS recognition and signal
 transduction, and possible therapeutic application of monoclonal
 antibodies inhibiting TLR4/MD-2 functions.
 CC 14-0 (Mammalian Pathological Biochemistry)
 Section cross-reference(s): 15
 ST review **endotoxin** shock TLR receptor lipopolysaccharide
 recognition
 IT Proteins
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (MD-2; **endotoxin** (lipopolysaccharide)
 recognition mechanism by Toll-like receptor (TLR) in relation to
endotoxin shock)
 IT Receptors
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (TLR (Toll-like receptor); **endotoxin** (lipopolysaccharide)
 recognition mechanism by Toll-like receptor (TLR) in relation to
endotoxin shock)
 IT Lipopolysaccharides
 RL: ADV (Adverse effect, including toxicity); BSU (Biological study,
 unclassified); BIOL (Biological study)
 (bacterial; **endotoxin** (lipopolysaccharide) recognition

mechanism by Toll-like receptor (TLR) in relation to **endotoxin shock**)

IT Molecular recognition
 (**endotoxin** (lipopolysaccharide) recognition mechanism by Toll-like receptor (TLR) in relation to **endotoxin shock**)

IT Toxins
 RL: ADV (Adverse effect, including toxicity); BSU (Biological study, unclassified); BIOL (Biological study)
 (**endotoxins**; **endotoxin** (lipopolysaccharide) recognition mechanism by Toll-like receptor (TLR) in relation to **endotoxin shock**)

IT Shock (circulatory collapse)
 (septic; **endotoxin** (lipopolysaccharide) recognition mechanism by Toll-like receptor (TLR) in relation to **endotoxin shock**)

L53 ANSWER 10 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN
 ACCESSION NUMBER: 2004:70743 CAPLUS
 DOCUMENT NUMBER: 140:355309
 TITLE: **Endotoxin** recognition molecules, Toll-like receptor 4-**MD-2**
 AUTHOR(S): Miyake, Kensuke
 CORPORATE SOURCE: The Institute of Medical Science, Department of Microbiology and Immunology, Division of Infectious Genetics, The University of Tokyo, 4-6-1 Shirokanedai, Tokyo, 108-8639, Japan
 SOURCE: Seminars in Immunology (2004), 16(1), 11-16
 CODEN: SEIME2; ISSN: 1044-5323
 PUBLISHER: Elsevier Science B.V.
 DOCUMENT TYPE: Journal; General Review
 LANGUAGE: English
 ED Entered STN: 29 Jan 2004
 AB A review. Toll-like receptors (TLRs) are innate pathogen recognition mols. for microbial products. Lipopolysaccharide (LPS), a membrane constituent of Gram-neg. bacteria, is one of the most potent microbial products. LPS is recognized by TLR4 and MD-2. TLR4 is a transmembrane protein, the extracellular domain of which is composed of a protein motif called leucine-rich repeats (LRR). MD-2 is an extracellular mol. that is associated with the extracellular LRR of TLR4. MD-2 has a role in cell surface expression of TLR4 and interaction with LPS. TLR4-MD-2 contributes to containment of infections by Gram-neg. bacteria by activating immune responses.
 CC 15-0 (Immunochemistry)
 ST review **endotoxin** TLR4 receptor complex MD2 protein
 IT Proteins
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (**MD-2**, complexes, with TLR-4; in immune recognition of bacterial **endotoxin**)
 IT Receptors
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (TLR-4 (Toll-like receptor-4), complexes, with **MD-2**; in immune recognition of bacterial **endotoxin**)
 IT Lipopolysaccharides
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (bacterial; TLR-4 receptor/**MD-2** accessory protein in immune recognition of)
 IT Toxins
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (**endotoxins**; TLR-4 receptor/**MD-2** accessory protein in immune recognition of)
 IT Immunity

(innate; TLR-4 receptor/**MD-2** accessory protein in
recognition of bacterial **endotoxin**)

REFERENCE COUNT: 57 THERE ARE 57 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L53 ANSWER 11 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN
 ACCESSION NUMBER: 2004:683145 CAPLUS
 DOCUMENT NUMBER: 142:296120
 TITLE: Molecular mechanism of **endotoxin** (LPS)
 recognition
 AUTHOR(S): Miyake, Kensuke
 CORPORATE SOURCE: Institute of Medical Science, University of Tokyo,
 Japan
 SOURCE: Endotokishin Kenkyu (2003), 6, 23-30
 CODEN: EKNEBO
 PUBLISHER: Igaku Tosho Shuppan K.K.
 DOCUMENT TYPE: Journal; General Review
 LANGUAGE: Japanese
 ED Entered STN: 23 Aug 2004
 AB A review. The topics discussed are (1) processing of lipopolysaccharide (LPS); (2) Toll-like receptor 4 (TLR4) in the recognition of LPS; (3) MD-2 binding to TLR4; (4) MD-2 required for cell surface expression of TLR4; (5) role of MD-2 in TLR4 recognition of LPS; and (6) RP105-MD-1 complex in B cell recognition of LPS.
 CC 15-0 (Immunochemistry)
 ST review Toll like receptor TLR4 MD2 **endotoxin** lipopolysaccharide
 IT Proteins
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (**MD-2**, complexes with TLR4; Toll-like
 receptor 4-**MD-2** complex in recognition of
endotoxin)
 IT Receptors
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (TLR-4 (Toll-like receptor-4), complexes with **MD-**
 2; Toll-like receptor 4-**MD-2**
 complex in recognition of **endotoxin**)
 IT Molecular association
 Molecular recognition
 (Toll-like receptor 4-**MD-2** complex in
 recognition of **endotoxin**)
 IT Lipopolysaccharides
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (bacterial; Toll-like receptor 4-**MD-2**
 complex in recognition of **endotoxin**)
 IT Immunity
 (innate; Toll-like receptor 4-**MD-2** complex
 in recognition of **endotoxin**)

L53 ANSWER 12 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN
 ACCESSION NUMBER: 2002:588215 CAPLUS
 DOCUMENT NUMBER: 138:70580
 TITLE: TLR4-MD2 signaling pathway induced by
endotoxin
 AUTHOR(S): Li, Yongwang; Ma, Li; Mao, Baoling; Qian, Guisheng
 CORPORATE SOURCE: Institute of Respiratory Disease, Xinqiao Hospital,
 the Third Military Medical University, Chungking,
 400037, Peop. Rep. China
 SOURCE: Zhongguo Yaolixue Tongbao (2002), 18(2), 121-125
 CODEN: ZYTOE8; ISSN: 1001-1978
 PUBLISHER: Anhui Yike Daxue Linchuan Yaoli Yanjiuso

DOCUMENT TYPE: Journal; General Review
 LANGUAGE: Chinese
 ED Entered STN: 08 Aug 2002
 AB A review with 24 refs. on TLR4-MD2 (TLR4 = toll-like receptor-4) signaling pathway induced by endotoxin with subdivision headings: (1) survey on TLRs; (2) role of TLR4 and its accessory protein MD2 in signaling pathway; (3) basic composition of lipopolysaccharide (LPS) signaling pathway mediated by TLR4-MD2; Biol. significance of TLR4-MD2 signaling pathway deficiency; (5) expression and role of TLR4-MD2 in different tissues; and (6) conclusions.
 CC 14-0 (Mammalian Pathological Biochemistry)
 ST review TLR4 MD2 signaling pathway **endotoxin** inflammation
 IT Proteins
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (**MD-2, complex** with TLR-4; TLR4- MD2
 signaling pathway induced by **endotoxin**)
 IT Transcription factors
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (**NF-κB** (nuclear factor of κ light chain gene enhancer in
 B-cells); TLR4- MD2 signaling pathway induced by **endotoxin**)
 IT Receptors
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (**TLR-4 (Toll-like receptor-4), complex** with **MD-**
 2; TLR4- MD2 signaling pathway induced by endotoxin)
 IT Inflammation
 Signal transduction, biological
 (**TLR4- MD2 signaling pathway induced by endotoxin**)
 IT Lipopolysaccharides
 RL: ADV (Adverse effect, including toxicity); BIOL (Biological study)
 (**TLR4- MD2 signaling pathway induced by endotoxin**)
 IT CD14 (antigen)
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (**TLR4- MD2 signaling pathway induced by endotoxin**)
 IT Toxins
 RL: ADV (Adverse effect, including toxicity); BIOL (Biological study)
 (**endotoxins; TLR4- MD2 signaling pathway induced by endotoxin**)

L53 ANSWER 13 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN
 ACCESSION NUMBER: 2003:115771 CAPLUS
 DOCUMENT NUMBER: 138:335921
 TITLE: Role of glucosylation of **MD-2** in
 endotoxin signal transduction
 AUTHOR(S): Onishi, Takahiro; Muroi, Masashi; Tanamoto, Kenichi
 CORPORATE SOURCE: Division of Microorganisms, National Institute of
 Health Sciences, Japan
 SOURCE: Endotokishin Kenkyu (2002), 5, 50-55
 CODEN: EKNEBO
 PUBLISHER: Igaku Tosho Shuppan K.K.
 DOCUMENT TYPE: Journal; General Review
 LANGUAGE: Japanese
 ED Entered STN: 14 Feb 2003
 AB A review discusses role of glucosylation of **MD-2**
 protein in signaling of **endotoxin** in monocytes, and involvement
 of TLR4 receptor.
 CC 15-0 (Immunochemistry)
 ST review glucosylation MD2 protein **endotoxin** signaling
 IT Proteins
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (**MD-2; role of glucosylation of MD-**
 2 in endotoxin signal transduction)

IT Receptors
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (TLR-4 (Toll-like receptor-4); role of glucosylation of MD-
 2 in endotoxin signal transduction)

IT Glucosylation
 Human
 Macrophage
 Signal transduction, biological
 (role of glucosylation of MD-2 in endotoxin
 signal transduction)

IT CD14 (antigen)
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (role of glucosylation of MD-2 in endotoxin
 signal transduction)

L53 ANSWER 14 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2001:491171 CAPLUS

DOCUMENT NUMBER: 136:149763

TITLE: Molecular genetic analysis of an endotoxin
 nonresponder mutant cell line: a point mutation in a
 conserved region of MD-2 abolishes
 endotoxin-induced signaling

AUTHOR(S): Schromm, Andra B.; Lien, Egil; Henneke, Philipp; Chow,
 Jesse C.; Yoshimura, Atsutoshi; Heine, Holger; Latz,
 Eicke; Monks, Brian G.; Schwartz, David A.; Miyake,
 Kensuke; Golenbock, Douglas T.

CORPORATE SOURCE: Evans Biomedical Research Center, Boston University
 School of Medicine, Boston, MA, 02118, USA

SOURCE: Journal of Experimental Medicine (2001), 194(1), 79-88
 CODEN: JEMEA; ISSN: 0022-1007

PUBLISHER: Rockefeller University Press

DOCUMENT TYPE: Journal

LANGUAGE: English

ED Entered STN: 08 Jul 2001

AB Somatic cell mutagenesis is a powerful tool for characterizing receptor systems. We reported previously two complementation groups of mutant cell lines derived from CD14-transfected Chinese hamster ovary-K1 fibroblasts defective in responses to bacterial endotoxin. Both classes of mutants expressed a normal gene product for Toll-like receptor (TLR)4, and fully responded to stimulation by tumor necrosis factor (TNF)- α or interleukin (IL)-1 β . We identified the lesion in one of the complementation groups in the gene for MD-2, a putative TLR4 coreceptor. The nonresponder phenotype of this mutant was reversed by transfection with MD-2. Cloning of MD-2 from the nonresponder cell line revealed a point mutation in a highly conserved region resulting in a C95Y amino acid exchange. Both forms of MD-2 colocalized with TLR4 on the cell surface after transfection, but only the wild-type cDNA reverted the lipopolysaccharide (LPS) nonresponder phenotype. Furthermore, soluble MD-2, but not soluble MD-2C95Y, functioned to enable LPS responses in cells that expressed TLR4. Thus, MD-2 is a required component of the LPS signaling complex and can function as a soluble receptor for cells that do not otherwise express it. We hypothesize that MD-2 conformationally affects the extracellular domain of TLR4, perhaps resulting in a change in affinity for LPS or functioning as a portion of the true ligand for TLR4.

CC 15-10 (Immunochemistry)

ST MD2 Toll like receptor endotoxin signaling; lipopolysaccharide
 signaling MD2 TLR4

IT Signal transduction, biological

(LPS signaling, MD-2 is required component of;
 point mutation in conserved region of MD-2

abolishes endotoxin-induced signaling)

IT Lipopolysaccharides
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (LPS, MD-2 is required component of LPS signaling
 complex; point mutation in conserved region of MD-
 2 abolishes endotoxin-induced signaling)

IT Proteins
 RL: BSU (Biological study, unclassified); PRP (Properties); BIOL
 (Biological study)
 (MD-2, TLR4 coreceptor; point mutation in conserved
 region of MD-2 abolishes endotoxin
 -induced signaling)

IT Receptors
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (TLR-4 (Toll-like receptor-4), MD-2 colocalized
 with; point mutation in conserved region of MD-2
 abolishes endotoxin-induced signaling)

IT Protein motifs
 (conserved region, of MD-2, C95Y substitution in;
 point mutation in conserved region of MD-2
 abolishes endotoxin-induced signaling)

IT Toxins
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (endotoxins, LPS; point mutation in conserved region of
 MD-2 abolishes endotoxin-induced signaling)

IT Gram-negative bacteria
 (point mutation in conserved region of MD-2
 abolishes endotoxin-induced signaling)

IT Mutation
 (point; point mutation in conserved region of MD-2
 abolishes endotoxin-induced signaling)

REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L53 ANSWER 15 OF 22 MEDLINE on STN DUPLICATE 3
 ACCESSION NUMBER: 2005211589 MEDLINE
 DOCUMENT NUMBER: PubMed ID: 15845500
 TITLE: Differential induction of the toll-like receptor
 4-MyD88-dependent and -independent signaling pathways by
 endotoxins.
 AUTHOR: Zughaier Susu M; Zimmer Shanta M; Datta Anup; Carlson
 Russell W; Stephens David S
 CORPORATE SOURCE: Division of Infectious Diseases, Emory University School of
 Medicine, VAMC (I-151), 1670 Clairmont Rd, Atlanta, GA
 30033, USA.. szughai@emory.edu
 CONTRACT NUMBER: R01 AI033517-10 (NIAID)
 SOURCE: Infection and immunity, (2005 May) Vol. 73, No. 5, pp.
 2940-50.
 Journal code: 0246127. ISSN: 0019-9567.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 200506
 ENTRY DATE: Entered STN: 23 Apr 2005
 Last Updated on STN: 8 Jun 2005
 Entered Medline: 7 Jun 2005

AB The biological response to endotoxin mediated through the Toll-like receptor 4 (TLR4) -MD-2 receptor complex is directly related to lipid A structure or configuration. Endotoxin structure may also influence activation of the MyD88-dependent and -independent signaling pathways of TLR4. To address this possibility, human macrophage-like cell lines (THP-1, U937, and MM6) or murine macrophage RAW 264.7 cells were stimulated with picomolar concentrations of highly purified endotoxins. Harvested supernatants from previously stimulated cells were also used to stimulate RAW 264.7 or 23ScCr (TLR4-deficient) macrophages (i.e., indirect induction). Neisseria meningitidis lipooligosaccharide (LOS) was a potent direct inducer of the MyD88-dependent pathway molecules tumor necrosis factor alpha (TNF-alpha), interleukin-1beta (IL-1beta), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 3alpha (MIP-3alpha), and the MyD88-independent molecules beta interferon (IFN-beta), nitric oxide, and IFN-gamma-inducible protein 10 (IP-10). Escherichia coli 55:B5 and Vibrio cholerae lipopolysaccharides (LPSs) at the same pmole/ml lipid A concentrations induced comparable levels of TNF-alpha, IL-1beta, and MIP-3alpha, but significantly less IFN-beta, nitric oxide, and IP-10. In contrast, LPS from Salmonella enterica serovars Minnesota and Typhimurium induced amounts of IFN-beta, nitric oxide, and IP-10 similar to meningococcal LOS but much less TNF-alpha and MIP-3alpha in time course and dose-response experiments. No MyD88-dependent or -independent response to endotoxin was seen in TLR4-deficient cell lines (C3H/HeJ and 23ScCr) and response was restored in TLR4-MD-2-transfected human embryonic kidney 293 cells. Blocking the MyD88-dependent pathway by DNMyD88 resulted in significant reduction of TNF-alpha release but did not influence nitric oxide release. IFN-beta polyclonal antibody and IFN-alpha/beta receptor 1 antibody significantly reduced nitric oxide release. N. meningitidis endotoxin was a potent agonist of both the MyD88-dependent and -independent signaling pathways of the TLR4 receptor complex of human macrophages. E. coli 55:B5 and Vibrio cholerae LPS, at the same picomolar lipid A concentrations, selectively induced the MyD88-dependent pathway, while Salmonella LPS activated the MyD88-independent pathway.

CT Adaptor Proteins, Signal Transducing
 Animals
 *Antigens, Differentiation: ME, metabolism
 Cell Line
 Cytokines: ME, metabolism
 *Endotoxins: CH, chemistry
 *Endotoxins: PH, physiology
 Gram-Negative Bacteria: IM, immunology
 Gram-Negative Bacteria: ME, metabolism
 Gram-Negative Bacteria: PY, pathogenicity
 Humans
 Lipid A: CH, chemistry
 Lipid A: PD, pharmacology
 Lipopolysaccharides: CH, chemistry
 Macrophage Activation: DE, drug effects
 *Macrophage Activation: IM, immunology
 Macrophages: IM, immunology
 Macrophages: ME, metabolism
 *Membrane Glycoproteins: ME, metabolism
 Mice
 Mice, Inbred C3H
 Nitric Oxide: ME, metabolism
 *Receptors, Cell Surface: ME, metabolism
 *Receptors, Immunologic: ME, metabolism
 Research Support, N.I.H., Extramural

Research Support, U.S. Gov't, P.H.S.
 *Signal Transduction
 Toll-Like Receptor 4
 Toll-Like Receptors

L53 ANSWER 16 OF 22 MEDLINE on STN DUPLICATE 5
 ACCESSION NUMBER: 2005303694 MEDLINE
 DOCUMENT NUMBER: PubMed ID: 15949133
 TITLE: Detoxifying endotoxin: time, place and person.
 AUTHOR: Munford Robert S
 CORPORATE SOURCE: Molecular Host Defense Laboratory, Departments of Internal Medicine and Microbiology, University of Texas Southwestern Medical School, Dallas, Texas 75390, USA..
 robert.munford@utsouthwestern.edu
 CONTRACT NUMBER: AI8188 (NIAID)
 SOURCE: Journal of endotoxin research, (2005) Vol. 11, No. 2, pp. 69-84. Ref: 166
 Journal code: 9433350. ISSN: 0968-0519.
 PUB. COUNTRY: England: United Kingdom
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 General Review; (REVIEW)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 200508
 ENTRY DATE: Entered STN: 14 Jun 2005
 Last Updated on STN: 3 Aug 2005
 Entered Medline: 2 Aug 2005

AB Animals that cannot sense endotoxin may die if they are infected by Gram-negative bacteria. Animals that sense endotoxin and respond too vigorously may also die, victims of their own inflammatory reactions. The outcome of Gram-negative bacterial infection is thus determined not only by an individual's ability to sense endotoxin and respond to its presence, but also by numerous phenomena that inactivate endotoxin and/or prevent harmful reactions to it. Endotoxin sensing requires the MD-2/TLR4 recognition complex and occurs principally in local tissues and the liver. This review highlights the known detoxification mechanisms, which include: (i) proteins that facilitate LPS sequestration by plasma lipoproteins, prevent interactions between the bioactive lipid A moiety and MD-2/TLR4, or promote cellular uptake via non-signaling pathway(s); (ii) enzymes that deacylate or dephosphorylate lipid A; (iii) mechanisms that remove LPS and Gram-negative bacteria from the bloodstream; and (iv) neuroendocrine adaptations that modulate LPS-induced mediator production or neutralize pro-inflammatory molecules in the circulation. In general, the mechanisms for sensing and detoxifying endotoxin seem to be compartmentalized (local versus systemic), dynamic, and variable between individuals. They may have evolved to confine infection and inflammation to extravascular sites of infection while preventing harmful systemic reactions. Integration of endotoxin sensing and detoxification is essential for successful host defense.

CT Animals
 Bacterial Infections: ME, metabolism
 *Endotoxins: ME, metabolism
 Endotoxins: TO, toxicity
 Humans
 Lipid A: ME, metabolism
 Research Support, N.I.H., Extramural
 Research Support, U.S. Gov't, P.H.S.
 Reticuloendothelial System: ME, metabolism

L53 ANSWER 17 OF 22 MEDLINE on STN DUPLICATE 7
 ACCESSION NUMBER: 2004602663 MEDLINE
 DOCUMENT NUMBER: PubMed ID: 15472123
 TITLE: Potential role of endotoxin as a proinflammatory mediator of atherosclerosis.
 AUTHOR: Stoll Lynn L; Denning Gerene M; Weintraub Neal L
 CORPORATE SOURCE: Department of Internal Medicine, Division of Cardiovascular Diseases, University of Iowa, Iowa City and The VA Medical Center, IA 52242, USA.. stoll1@mail.medicine.uiowa.edu
 SOURCE: Arteriosclerosis, thrombosis, and vascular biology, (2004 Dec) Vol. 24, No. 12, pp. 2227-36. Electronic Publication: 2004-10-07. Ref: 175
 Journal code: 9505803. E-ISSN: 1524-4636.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: General Review; (REVIEW)
 ENTRY MONTH: Priority Journals
 200506
 ENTRY DATE: Entered STN: 4 Dec 2004
 Last Updated on STN: 22 Jun 2005
 Entered Medline: 21 Jun 2005

AB Atherosclerosis is increasingly recognized as a chronic inflammatory disease. Although a variety of inflammatory markers (ie, C-reactive protein) have been associated with atherosclerosis and its consequences, it is important to identify principal mediators of the inflammatory responses. One potentially important source of vascular inflammation in atherosclerosis is bacterial endotoxin. Mutations in Toll-like receptor 4 (TLR-4), an integral component of the endotoxin signaling complex, are fairly common in the Caucasian population and have recently been associated with reduced incidence of atherosclerosis and other cardiovascular diseases in some studies. Moreover, epidemiological studies suggest that endotoxemia at levels as low as 50 pg/mL constitutes a strong risk factor for the development of atherosclerosis. Endotoxin concentrations in this range may be produced by a variety of common subclinical Gram-negative infections. In this article, we outline the main elements of the **endotoxin** signaling receptor **complex** that initiates proinflammatory signaling (lipopolysaccharide binding protein [LBP], CD14, TLR-4, and **MD-2**) and discuss how changes in expression of these molecules may affect proatherogenic responses in the vessel wall. We also describe some of the proinflammatory effects of endotoxin that may be relevant to atherosclerosis, and discuss how serum lipoproteins, especially high-density lipoprotein, may modulate endotoxin-induced inflammatory responses. Further, we discuss recent findings suggesting that the lipid-lowering statins may have an additional protective role in blocking at least some of these proinflammatory signaling pathways. Finally, we discuss species diversity with regard to endotoxin signaling that should be considered when extrapolating experimental data from animal models to humans.

CT Animals
 *Arteriosclerosis: PA, pathology
 *Endotoxins: PH, physiology
 Humans
 Inflammation: ME, metabolism

L53 ANSWER 18 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
 ACCESSION NUMBER: 2004:72508 BIOSIS
 DOCUMENT NUMBER: PREV200400076152

TITLE: Lysines 128 and 132 enable lipopolysaccharide binding to MD-2, leading to Toll-like receptor-4 aggregation and signal transduction.

AUTHOR(S): Visintin, Alberto; Latz, Eicke; Monks, Brian G.; Espevik, Terje; Golenbock, Douglas T. [Reprint Author]

CORPORATE SOURCE: Dept. of Medicine, University of Massachusetts Medical School, 364 Plantation St., LRB 309, Worcester, MA, 01605, USA
douglas.golenbock@umassmed.edu

SOURCE: Journal of Biological Chemistry, (November 28 2003) Vol. 278, No. 48, pp. 48313-48320. print.
CODEN: JBCHA3. ISSN: 0021-9258.

DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 4 Feb 2004
Last Updated on STN: 4 Feb 2004

AB Three cell-surface proteins have been recognized as components of the mammalian signaling receptor for bacterial lipopolysaccharide (LPS): CD14, Toll-like receptor-4 (TLR4), and MD-2. Biochemical and visual studies shown here demonstrate that the role of CD14 in signal transduction is to enhance LPS binding to MD-2, although its expression is not essential for cellular activation. These studies clarify how MD-2 functions: we found that MD-2 enables TLR4 binding to LPS and allows the formation of stable receptor complexes. MD-2 must be bound to TLR4 on the cell surface before binding can occur. Consequently, TLR4 clusters into receptosomes (many of which are massive) that recruit intracellular toll/IL-1/resistance domain-containing adapter proteins within minutes, thus initiating signal transduction. TLR4 activation correlates with the ability of MD-2 to bind LPS, as MD-2 mutants that still bind TLR4, but are impaired in the ability to bind LPS, conferred a greatly blunted LPS response. These findings help clarify the earliest events of TLR4 triggering by LPS and identify MD-2 as an attractive target for pharmacological intervention in endotoxin-mediated diseases.

IT Major Concepts
Biochemistry and Molecular Biophysics; Cell Biology; Immune System (Chemical Coordination and Homeostasis); Metabolism

IT Chemicals & Biochemicals
CD14: cell-surface protein, signal transduction; MD-2: cell-surface protein, lipopolysaccharide binding; Toll-like receptor-4: aggregation, cell-surface protein, signal transduction; lipopolysaccharide; lysine 128; lysine 132

L53 ANSWER 19 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2003:521649 BIOSIS
DOCUMENT NUMBER: PREV200300510588

TITLE: Lipopolysaccharide interaction with cell surface toll-like receptor 4-MD-2: Higher affinity than that with MD-2 or CD14.

AUTHOR(S): Akashi, Sachiko; Saitoh, Shin-ichiroh; Wakabayashi, Yasutaka; Kikuchi, Takane; Takamura, Noriaki; Nagai, Yoshinori; Kusumoto, Yutaka; Fukase, Koichi; Kusumoto, Shoichi; Adachi, Yoshiyuki; Kosugi, Atsushi; Miyake, Kensuke [Reprint Author]

CORPORATE SOURCE: Division of Infectious Genetics, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minatoku, Tokyo, 108-8639, Japan
kmiyake@ims.u-tokyo.ac.jp

SOURCE: Journal of Experimental Medicine, (October 6 2003) Vol.

198, No. 7, pp. 1035-1042. print.
 ISSN: 0022-1007 (ISSN print).

DOCUMENT TYPE: Article
 LANGUAGE: English
 ENTRY DATE: Entered STN: 5 Nov 2003
 Last Updated on STN: 5 Nov 2003

AB Toll-like receptors (TLRs) are innate recognition molecules for microbial products, but their direct interactions with corresponding ligands remain unclarified. LPS, a membrane constituent of gram-negative bacteria, is the best-studied TLR ligand and is recognized by TLR4 and MD-2, a molecule associated with the extracellular domain of TLR4. Although TLR4-MD-2 recognizes LPS, little is known about the physical interaction between LPS and TLR4-MD-2. Here, we demonstrate cell surface LPS-TLR4-MD-2 complexes. CD14 greatly enhances the formation of LPS-TLR4-MD-2 complexes, but is not coprecipitated with LPS-TLR4-MD-2 complexes, suggesting a role for CD14 in LPS loading onto TLR4-MD-2 but not in the interaction itself between LPS and TLR4-MD-2. A tentative dissociation constant (Kd) for LPS-TLR4-MD-2 complexes was apprx3 nM, which is apprx10-20 times lower than the reported Kd for LPS-MD-2 or LPS-CD14. The presence of detergent disrupts LPS interaction with CD14 but not with TLR4-MD-2. E5531, a lipid A antagonist developed for therapeutic intervention of endotoxin shock, blocks LPS interaction with TLR4-MD-2 at a concentration 100 times lower than that required for blocking LPS interaction with CD14. These results reveal direct LPS interaction with cell surface TLR4-MD-2 that is distinct from that with MD-2 or CD14.

IT Major Concepts
 Biochemistry and Molecular Biophysics; Immune System (Chemical Coordination and Homeostasis)
 IT Parts, Structures, & Systems of Organisms
 cell surface; macrophage: blood and lymphatics, immune system
 IT Diseases
 endotoxin shock: bacterial disease, infectious disease
 IT Chemicals & Biochemicals
 CD14; E5531; MD-2; Toll-like receptor 4 [TLR4]; Toll-like receptor 4-MD-2 [TLR4-MD2]; lipopolysaccharide [LPS]

L53 ANSWER 20 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2004:163318 BIOSIS
 DOCUMENT NUMBER: PREV200400164077
 TITLE: Regulation of interactions of endotoxin with host cells.
 AUTHOR(S): Gioannini, Theresa L.; Teghanemt, Athmane; Zaremba, Kol A.; Weiss, Jerrold P. [Reprint Author]
 CORPORATE SOURCE: Roy J. and Lucille A. Carver College of Medicine, 200 Hawkins Drive, Iowa City, IA, 52242, USA
 jerrold-weiss@uiowa.edu
 SOURCE: Journal of Endotoxin Research, (2003) Vol. 9, No. 6, pp. 401-408. print.
 ISSN: 0968-0519.

DOCUMENT TYPE: Article
 LANGUAGE: English
 ENTRY DATE: Entered STN: 24 Mar 2004
 Last Updated on STN: 24 Mar 2004

AB Potent Toll-like receptor 4 (TLR4)-dependent cell activation by endotoxin requires lipopolysaccharide-binding protein (LBP) and CD14-dependent delivery of endotoxin to cells containing MD-2 and TLR4. We have used metabolically labeled (¹⁴C) meningococcal lipooligosaccharide (LOS), purified recombinant endotoxin-binding proteins, and cultured endothelial cells to better

define protein:endotoxin intermediates key in cell activation in the absence of functional membrane (m) CD14. Protein:endotoxin complexes or aggregates (agg) were purified by gel sieving and characterized by immunocapture and bio-assays. Cell activation closely correlated with LBP, albumin and soluble (s) CD14-dependent conversion of endotoxin agg (Mr_{gtoreq}20X106) to monomeric (Mr_{apprx}55X103) endotoxin:SCD14 complexes. Ordered interaction of LBP (+albumin) and sCD14 with LOSagg was required for the efficient formation of a bioactive endotoxin:SCD14 complex and potent cell activation. Increasing the ratio of LBP/sCD14 or addition of bactericidal/permeability-increasing protein (BPI) reduced accumulation of endotoxin:SCD14 complexes and instead yielded aggregates of endotoxin (Mr_{apprx}1-20X106) containing LBP or BPI that were taken up by cells in a CD14- and TLR4-independent manner without inducing pro-inflammatory responses. These findings strongly suggest that host machinery linked to TLR4-dependent cellular activation or TLR4-independent cellular clearance of endotoxin selectively recognizes different protein:endotoxin complexes. At the outset of infection, the low concentrations of LBP present and absence of extracellular BPI favor formation of pro-inflammatory endotoxin:CD14 complexes. The mobilization of LBP and BPI that is triggered by inflammation directs endotoxin for clearance and hence resolution of endotoxin-triggered inflammation.

IT Major Concepts

Immune System (Chemical Coordination and Homeostasis); Infection;
Toxicology

IT Parts, Structures, & Systems of Organisms
cell

IT Chemicals & Biochemicals

CD14; TLR4 [toll-like receptor 4]; endotoxin: toxin; lipopolysaccharide binding protein [LBP]

L53 ANSWER 21 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2003:234536 BIOSIS

DOCUMENT NUMBER: PREV200300234536

TITLE: Overexpression of CD14, TLR4, and MD-2 in HEK 293T cells does not prevent induction of in vitro endotoxin tolerance.

AUTHOR(S): Medvedev, Andrei E.; Vogel, Stefanie N. [Reprint Author]

CORPORATE SOURCE: Department of Microbiology and Immunology, University of Maryland, 655 West Baltimore St, 13-009, Baltimore, MD, 21201, USA

svogel@som.umaryland.edu

SOURCE: Journal of Endotoxin Research, (2003) Vol. 9, No. 1, pp. 60-64. print.

ISSN: 0968-0519.

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 14 May 2003

Last Updated on STN: 14 May 2003

AB TLR4 and MD-2 are necessary for conferring cellular responsiveness to LPS. Prior exposure to LPS induces a transient state of cell refractoriness to subsequent LPS re-stimulation, known as 'endotoxin tolerance'. While induction of LPS tolerance has been reported to correlate with down-regulation of cell surface expression of TLR4/MD-2, other mechanisms of LPS tolerance have been revealed that target intracellular intermediates downstream of the TLR4/MD-2 complex. In this study, we sought to examine whether endotoxin tolerance could be induced under conditions where expression of TLR4 and MD-2 proteins is not affected by LPS. Human HEK 293T cells are

completely unresponsive to LPS, but acquire high LPS sensitivity following transient transfection with CD14, TLR4, and MD-2 (293T/CD14/TLR4/MD-2 cells), as judged by NF-kappaB activation, ERK 1/2 phosphorylation, and TNF-alpha gene expression. Prior exposure of 293T/CD14/TLR4/MD-2 cells to LPS resulted in a significant decrease of LPS-mediated responses, yet failed to affect expression levels of TLR4 and MD-2. Thus, altered expression and/or function of intracellular mediators downstream of the TLR4/MD-2 complex play an important role in mediating LPS tolerance.

IT Major Concepts

Immune System (Chemical Coordination and Homeostasis); Infection; Molecular Genetics (Biochemistry and Molecular Biophysics); Toxicology

IT Chemicals & Biochemicals

CD14: overexpression; ERK1; ERK2; LPS [lipopolysaccharide]: toxin; MD-2: overexpression; TLR4 [toll-like receptor 4]: overexpression; endotoxin: toxin

L53 ANSWER 22 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2003:5626 BIOSIS

DOCUMENT NUMBER: PREV200300005626

TITLE: Dysregulation of LPS-induced toll-like receptor 4-MyD88 complex formation and IL-1 receptor-associated kinase 1 activation in endotoxin-tolerant cells.

AUTHOR(S): Medvedev, Andrei E.; Lentschat, Arnd; Wahl, Larry M.; Golenbock, Douglas T.; Vogel, Stefanie N. [Reprint Author]

CORPORATE SOURCE: Department of Microbiology and Immunology, University of Maryland, Baltimore, MD, 21201-1559, USA svogel@som.umaryland.edu

SOURCE: Journal of Immunology, (November 1 2002) Vol. 169, No. 9, pp. 5209-5216. print.

ISSN: 0022-1767 (ISSN print).

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 18 Dec 2002

Last Updated on STN: 18 Dec 2002

AB Prior exposure to LPS induces a transient state of cell refractoriness to subsequent LPS restimulation, known as endotoxin tolerance. Induction of LPS tolerance has been reported to correlate with decreased cell surface expression of the LPS receptor complex, Toll-like receptor 4 (TLR4)/MD-2. However, other results have underscored the existence of mechanisms of LPS tolerance that operate downstream of TLR4/MD-2. In the present study we sought to delineate further the molecular basis of LPS tolerance by examining the TLR4 signaling pathway in endotoxin-tolerant cells. Pretreatment of human monocytes with LPS decreased LPS-mediated NF-kappaB activation, p38 mitogen-activated protein kinase phosphorylation, and TNF-alpha gene expression, documenting the induction of endotoxin tolerance. FACS and Western blot analyses of LPS-tolerant monocytes showed increased TLR2 expression, whereas TLR4 expression levels were not affected. Comparable levels of mRNA and protein for myeloid differentiation factor 88 (MyD88), IL-1R-associated kinase 1 (IRAK-1), and TNFR-associated factor-6 were found in normal and LPS-tolerant monocytes, while MD-2 mRNA expression was slightly increased in LPS-tolerant cells. LPS induced the association of MyD88 with TLR4 and increased IRAK-1 activity in medium-pretreated cells. In LPS-tolerant monocytes, however, MyD88 failed to be recruited to TLR4, and IRAK-1 was not activated in response to LPS stimulation. Moreover, endotoxin-tolerant CHO cells that overexpress human TLR4 and MD-2 also showed decreased IRAK-1 kinase activity in response to LPS despite the failure of LPS to inhibit cell surface expression of transfected TLR4 and MD

-2 proteins. Thus, decreased TLR4-MyD88 complex formation with subsequent impairment of IRAK-1 activity may underlie the LPS-tolerant phenotype.

IT Major Concepts

Blood and Lymphatics (Transport and Circulation); Immune System (Chemical Coordination and Homeostasis); Toxicology

IT Parts, Structures, & Systems of Organisms

monocytes: blood and lymphatics, immune system, in-vitro culture

IT Chemicals & Biochemicals

Toll-like receptor 4: endotoxin tolerant cell complexing dysregulation, entotoxin-induced myeloid differentiation factor 88 complexing; interleukin-1 receptor-associated kinase 1: defective endotoxin tolerant cell activation; lipopolysaccharide [endotoxin]: monocyte tolerance

L54 ANSWER 1 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 1

ACCESSION NUMBER: 2005:1207627 CAPLUS

DOCUMENT NUMBER: 143:458453

TITLE: Biochemical and Functional Characterization of Membrane Blebs Purified from Neisseria meningitidis Serogroup B

AUTHOR(S): Post, Deborah M. B.; Zhang, DeSheng; Eastvold, Joshua S.; Teghanemt, Athmane; Gibson, Bradford W.; Weiss, Jerrold P.

CORPORATE SOURCE: Inflammation Program, Department of Internal Medicine and the Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA, 52242, USA

SOURCE: Journal of Biological Chemistry (2005), 280(46), 38383-38394

CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Studies with purified aggregates of endotoxin have revealed the importance of lipopolysaccharide-binding protein (LBP)-dependent extraction and transfer of individual endotoxin mols. to CD14 in Toll-like receptor 4 (TLR4)-dependent cell activation. Endotoxin is normally embedded in the outer membrane of intact Gram-neg. bacteria and shed membrane vesicles ("blebs"). However, the ability of LBP and CD14 to efficiently promote TLR4-dependent cell activation by membrane-associated endotoxin has not been studied extensively. In this study, the authors used an acetate auxotroph of *Neisseria meningitidis* serogroup B to facilitate metabolic labeling of bacterial endotoxin and compared interactions of purified endotoxin aggregates and of membrane-associated endotoxin with LBP, CD14, and endotoxin-responsive cells. The endotoxin, phospholipid, and protein composition of the recovered blebs indicate that the blebs derive from the bacterial outer membrane. Proteomic anal. revealed an unusual enrichment in highly cationic ($pI > 9$) proteins. Both purified endotoxin aggregates and blebs activate monocytes and endothelial cells in a LBP-, CD14-, and TLR4/MD-2-dependent fashion, but the blebs were 3-10-fold less potent when normalized for the amount of endotoxin added. Differences in potency correlated with differences in efficiency of LBP-dependent delivery to and extraction of endotoxin by CD14. Both membrane phospholipids and endotoxin are extracted by LBP/soluble CD14 (sCD14) treatment, but only endotoxin ·sCD14 reacts with MD-2 and activates cells.

These findings indicate that the proinflammatory potency of endotoxin may be regulated not only by the intrinsic structural properties of endotoxin but also by its association with neighboring mols. in the outer membrane.

CT Human
 CT CD14 (antigen)
 CT Proteins
 CT Proteins
 CT Receptors
 CT Monocyte
 CT Proteins
 CT Blood vessel
 CT Neisseria meningitidis
 CT Lipopolysaccharides
 CT Proteins
 CT Cell activation
 CT Fatty acids, analysis
 CT Phospholipids, analysis
 CT Porins
 CT Endothelium
 CT Organelle

REFERENCE COUNT: 62 THERE ARE 62 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L54 ANSWER 2 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 2
 ACCESSION NUMBER: 2004:686516 CAPLUS
 DOCUMENT NUMBER: 141:308867
 TITLE: **Endotoxin responsiveness of human airway epithelia is limited by low expression of MD-2**
 AUTHOR(S): Jia, Hong Peng; Kline, Joel N.; Penisten, Andrea;
 Apicella, Michael A.; Gioannini, Theresa L.;
 Weiss, Jerrold; McCray, Paul B., Jr.
 CORPORATE SOURCE: Department of Pediatrics, Carver College of Medicine,
 University of Iowa and Iowa City Veterans
 Administration, Iowa City, IA, USA
 SOURCE: American Journal of Physiology (2004), 287(2, Pt. 1),
 L428-L437
 CODEN: AJPHAP; ISSN: 0002-9513
 PUBLISHER: American Physiological Society
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 AB The expression of inducible antimicrobial peptides, such as human β -defensin-2 (HBD-2) by epithelia, comprises a component of innate pulmonary defenses. We hypothesized that HBD-2 induction in airway epithelia is linked to pattern recognition receptors such as the Toll-like receptors (TLRs). We found that primary cultures of well-differentiated human airway epithelia express the mRNA for TLR-4, but little or no MD-2 mRNA, and display little HBD-2 expression in response to treatment with purified endotoxin + LPS binding protein (LBP) and soluble CD14. Expression of endogenous MD-2 by transduction of airway epithelial cells with an adenoviral vector encoding MD-2 or extracellular addition of recombinant MD-2 both increased the responses of airway epithelia to endotoxin + LBP and sCD14 by > 100-fold, as measured by NF- κ B-luciferase activity and HBD-2 mRNA expression. MD-2 mRNA could be induced in airway epithelia by exposure of these cells to specific bacterial or host products (e.g., killed Haemophilus influenzae, or TNF- α + IFN- γ). These findings suggest that MD-2, either coexpressed with TLR-4 or secreted when produced in excess of TLR-4 from neighboring cells, is required for airway epithelia to respond sensitively to endotoxin. The

regulation of MD-2 expression in airway epithelia and pulmonary macrophages may serve as a means to modify endotoxin responsiveness in the airway.

CT Proteins
 CT Receptors
 CT Human
 CT Toxins
 CT Respiratory system
 CT Epithelium

REFERENCE COUNT: 58 THERE ARE 58 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L54 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 3
 ACCESSION NUMBER: 2004:64598 CAPLUS
 DOCUMENT NUMBER: 140:269456
 TITLE: Regulation of interactions of endotoxin with host cells
 AUTHOR(S): Gioannini, Theresa L.; Teghanemt,
 Athmane; Zaremba, Kol A.; Weiss, Jerrold P.
 CORPORATE SOURCE: Departments of Internal Medicine, Division of Infectious Diseases and The Inflammation Program, Biochemistry, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA, USA
 SOURCE: Journal of Endotoxin Research (2003), 9(6), 401-408
 CODEN: JENREB; ISSN: 0968-0519
 PUBLISHER: Maney Publishing
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 AB Potent Toll-like receptor 4 (TLR4)-dependent cell activation by endotoxin requires lipopolysaccharide-binding protein (LBP) and CD14-dependent delivery of endotoxin to cells containing MD-2 and TLR4. We have used metabolically labeled [¹⁴C] meningococcal lipooligosaccharide (LOS), purified recombinant endotoxin-binding proteins, and cultured endothelial cells to better define protein:endotoxin intermediates key in cell activation in the absence of functional membrane (m) CD14. Protein:endotoxin complexes or aggregates (agg) were purified by gel sieving and characterized by immunocapture and bio-assays. Cell activation closely correlated with LBP, albumin and soluble (s) CD14-dependent conversion of endotoxin agg ($Mr \geq 20 + 106$) to monomeric ($Mr \text{ apprx. } 55 + 103$) endotoxin:sCD14 complexes. Ordered interaction of LBP (+ albumin) and sCD14 with LOSagg was required for the efficient formation of a bioactive endotoxin:sCD14 complex and potent cell activation. Increasing the ratio of LBP/sCD14 or addition of bactericidal/permeability-increasing protein (BPI) reduced accumulation of endotoxin:sCD14 complexes and instead yielded aggregates of endotoxin ($Mr \text{ apprx. } 1-20 + 106$) containing LBP or BPI that were taken up by cells in a CD14- and TLR4-independent manner without inducing pro-inflammatory responses. These findings strongly suggest that host machinery linked to TLR4-dependent cellular activation or TLR4-independent cellular clearance of endotoxin selectively recognizes different protein: endotoxin complexes. At the outset of infection, the low concns. of LBP present and absence of extracellular BPI favor formation of pro-inflammatory endotoxin:CD14 complexes. The mobilization of LBP and BPI that is triggered by inflammation directs endotoxin for clearance and hence resolution of endotoxin-triggered inflammation.

CT Proteins
 CT Cell activation
 CT Human
 CT Inflammation
 CT Molecular association

CT Receptors

CT Lipopolysaccharides

CT Blood vessel

CT Immunity

CT Proteins

CT Albumins, biological studies

CT CD14 (antigen)

CT Endothelium

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L54 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2005:426231 CAPLUS

DOCUMENT NUMBER: 142:480799

TITLE: Preparation of complexes of **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**

INVENTOR(S): Weiss, Jerrold P.; Gioannini, Theresa L.; Teghanemt, Athamane; Subramanian, Ramaswamy

PATENT ASSIGNEE(S): USA

SOURCE: U.S. Pat. Appl. Publ., 34 pp.

CODEN: USXXCO

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2005106179	A1	20050519	US 2003-715876	20031117
WO 2005049067	A1	20050602	WO 2004-US38375	20041117
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				

PRIORITY APPLN. INFO.: US 2003-715876 A 20031117

AB The disclosed invention provides purified water soluble complexes of **endotoxin** and **MD-2**. The invention also provides a method for making the complexes of the invention and a method for isolating complexes of the invention. Also provided are the method of using the complexes of the invention, e.g. method to increase or inhibit TLR4 receptor-dependent activation of cells by endotoxin in vitro or in vivo. Methods using complexes with mutant endotoxin are useful to decrease undesirable endotoxin-mediated inflammation. Methods using complexes with wild-type endotoxin are of use in promoting innate immunity and as immune adjuvants. The results of one example demonstrate that in primary cultures of human airway epithelia TLR4, but little or no MD-2 is expressed, so the cells are relatively unresponsive to added endotoxin. However, the cell responsiveness to endotoxin is markedly amplified by either the endogenous expression or exogenous addition of MD-2.

CT CD14 (antigen)

CT Animal cell line
 CT Proteins
 CT Receptors
 CT Immunostimulants
 CT Glycolipids
 CT Drug delivery systems
 CT Toxins
 CT Toxins
 CT Respiratory system
 CT Immunity
 CT Cell activation
 CT Escherichia
 CT Escherichia coli
 CT Francisella
 CT Francisella tularensis
 CT Haemophilus
 CT Haemophilus influenzae
 CT Neisseria
 CT Neisseria meningitidis
 CT Pseudomonas
 CT Pseudomonas aeruginosa
 CT Salmonella
 CT Salmonella typhimurium
 CT Anti-inflammatory agents
 CT Human
 CT Epithelium

L54 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2005:1016771 CAPLUS

DOCUMENT NUMBER: 143:304613

TITLE: Molecular Basis of Reduced Potency of Underacylated Endotoxins

AUTHOR(S): Teghanemt, Athmane; Zhang, DeSheng; Levis, Erika N.; Weiss, Jerrold P.; Gioannini, Theresa L.

CORPORATE SOURCE: Inflammation Program, Department of Internal Medicine, Coralville, IA, 52241, USA

SOURCE: Journal of Immunology (2005), 175(7), 4669-4676
CODEN: JOIMA3; ISSN: 0022-1767

PUBLISHER: American Association of Immunologists

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Potent TLR4-dependent cell activation by Gram-neg. bacterial endotoxins depends on sequential endotoxin-protein and protein-protein interactions with LPS-binding protein, CD14, myeloid differentiation protein 2 (MD-2), and TLR4. Previous studies have suggested that reduced agonist potency of underacylated endotoxins (i.e., tetra- or penta- vs. hexa-acylated) is determined by post-CD14 interactions. To better define the mol. basis of the differences in agonist potency of endotoxins differing in fatty acid acylation, the authors compared endotoxins (lipooligosaccharides (LOS)) from hexa-acylated wild-type (wt), penta-acylated mutant msbB meningococcal strains as well as tetra-acylated LOS generated by treatment of wt LOS with the deacylating enzyme, acyloxyacylhydrolase. To facilitate assay of endotoxin:protein and endotoxin:cell interactions, the endotoxins were purified after metabolic labeling with [3H]- or [14C]acetate. All LOS species tested formed monomeric complexes with MD-2 in an LPS-binding protein- and CD14-dependent manner with similar efficiency. However, msbB LOS:MD-2 and acyloxyacylhydrolase-treated LOS:MD-2 were at least 10-fold less potent in inducing TLR4-dependent cell activation than wt LOS:MD-2 and partially antagonized the action of wt

LOS:MD-2. These findings suggest that underacylated endotoxins produce decreased TLR4-dependent cell activation by altering the interaction of the **endotoxin:MD-2** complex with TLR4 in a way that reduces receptor activation. Differences in potency among these endotoxin species is determined not by different aggregate properties, but by different properties of monomeric **endotoxin:MD-2** complexes.

CT Proteins
 CT Receptors
 CT Toxins
 CT Lipopolysaccharides
 CT Structure-activity relationship
 CT Cell activation
 CT CD14 (antigen)

REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L54 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN
 ACCESSION NUMBER: 2005:489362 CAPLUS
 DOCUMENT NUMBER: 143:95745
 TITLE: Monomeric **endotoxin:protein** complexes are essential for TLR4-dependent cell activation
 AUTHOR(S): Gioannini, T. L.; Teghanemt, A.; Zhang, De S.; Levis, E. N.; Weiss, J. P.
 CORPORATE SOURCE: Department of Internal Medicine, Roy J. and Lucille A. Carver College of Medicine, University of Iowa and the Veterans' Administration Medical Center, Iowa City, IA, USA
 SOURCE: Journal of Endotoxin Research (2005), 11(2), 117-123
 CODEN: JENREB; ISSN: 0968-0519
 PUBLISHER: Maney Publishing
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 AB Potent TLR4-dependent cell activation by Gram-neg. bacterial endotoxin depends on sequential endotoxin-protein and protein-protein interactions with LBP, CD14, MD-2 and TLR4. LBP and CD14 combine, in an albumin-dependent fashion, to extract single endotoxin mols. from purified endotoxin aggregates (Eagg) or the bacterial outer membrane and form monomeric **endotoxin:CD14** complexes that are the preferred presentation of **endotoxin** for transfer to **MD-2**. Endotoxin in **endotoxin:CD14** is readily transferred to **MD-2**, again in an albumin-dependent manner, to form monomeric **endotoxin:MD-2** complex. This monomeric **endotoxin:protein** complex (**endotoxin:MD-2**) activates TLR4 at picomolar concns., independently of albumin, and is, therefore, the apparent ligand in endotoxin-dependent TLR4 activation. Tetra-, penta-, and hexa-acylated forms of meningococcal endotoxin (LOS) react similarly with LBP, CD14, and **MD-2** to form **endotoxin:MD-2** complexes. However, tetra- and penta-acylated LOS:MD-2 complexes are less potent TLR4 agonists than hexa-acylated LOS:MD-2. This is mirrored in the reduced activity of tetra-, penta- vs. hexa-acylated LOS aggregates (LOSagg) + LBP toward cells containing mCD14, **MD-2**, and TLR4. Therefore, changes in agonist potency of under-acylated meningococcal LOS are determined by differences in properties of monomeric **endotoxin:MD-2**.

CT Proteins
 CT Receptors
 CT Blood vessel
 CT Toxins
 CT Glycolipids

CT Cell activation
 CT CD14 (antigen)
 CT Albumins, biological studies
 CT Endothelium

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L54 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN
 ACCESSION NUMBER: 2004:292853 CAPLUS
 DOCUMENT NUMBER: 140:401625
 TITLE: Isolation of an **endotoxin-MD-2** complex that produces Toll-like receptor 4-dependent cell activation at picomolar concentrations
 AUTHOR(S): Gioannini, Theresa L.; Teghanemt, Athmane; Zhang, DeSheng; Coussens, Nathan P.; Dockstader, Wendie; Ramaswamy, S.; Weiss, Jerrold P.
 CORPORATE SOURCE: Inflammation Program, Department of Internal Medicine, and Department of Biochemistry Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Veterans Affairs Medical Center, Iowa City, IA, 52242, USA
 SOURCE: Proceedings of the National Academy of Sciences of the United States of America (2004), 101(12), 4186-4191
 CODEN: PNASA6; ISSN: 0027-8424
 PUBLISHER: National Academy of Sciences
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB Host proinflammatory responses to minute amts. of endotoxins derived from many Gram-neg. bacteria require the interaction of lipopolysaccharide-binding protein (LBP), CD14, Toll-like receptor 4 (TLR4) and MD-2. Optimal sensitivity to endotoxin requires an ordered series of endotoxin-protein and protein-protein interactions. At substoichiometric concns., LBP facilitates delivery of endotoxin aggregates to soluble CD14 (sCD14) to form monomeric endotoxin-sCD14 complexes. Subsequent interactions of endotoxin-sCD14 with TLR4 and/or MD-2 have not been specifically defined. This study reports the purification of a stable, monomeric, bioactive **endotoxin-MD-2** complex generated by treatment of **endotoxin-sCD14** with recombinant MD-2. Efficient generation of this complex occurred at picomolar concns. of endotoxin and nanogram per mL doses of MD-2 and required presentation of **endotoxin** to MD-2 as a monomeric **endotoxin-CD14** complex. TLR4-dependent delivery of endotoxin to human embryonic kidney (HEK) cells and cell activation at picomolar concns. of endotoxin occurred with the purified **endotoxin-MD-2** complex, but not with purified endotoxin aggregates with or without LBP and/or sCD14. The presence of excess MD-2 inhibited delivery of **endotoxin-MD-2** to HEK/TLR4 cells and cell activation. These findings demonstrate that TLR4-dependent activation of host cells by picomolar concns. of endotoxin occurs by sequential interaction and transfer of **endotoxin** to LBP, CD14, and MD-2 and simultaneous engagement of **endotoxin** and TLR4 by MD-2.

CT Animal cell line
 CT Proteins
 CT Proteins
 CT Receptors
 CT CD14 (antigen)
 CT Toxins

AUDET 10/715,876

CT Human
REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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